TOOLS, TECHNIQUES, AND STRATEGIES FOR TEACHING IN A REAL-WORLD CONTEXT WITH MICROBIOLOGY

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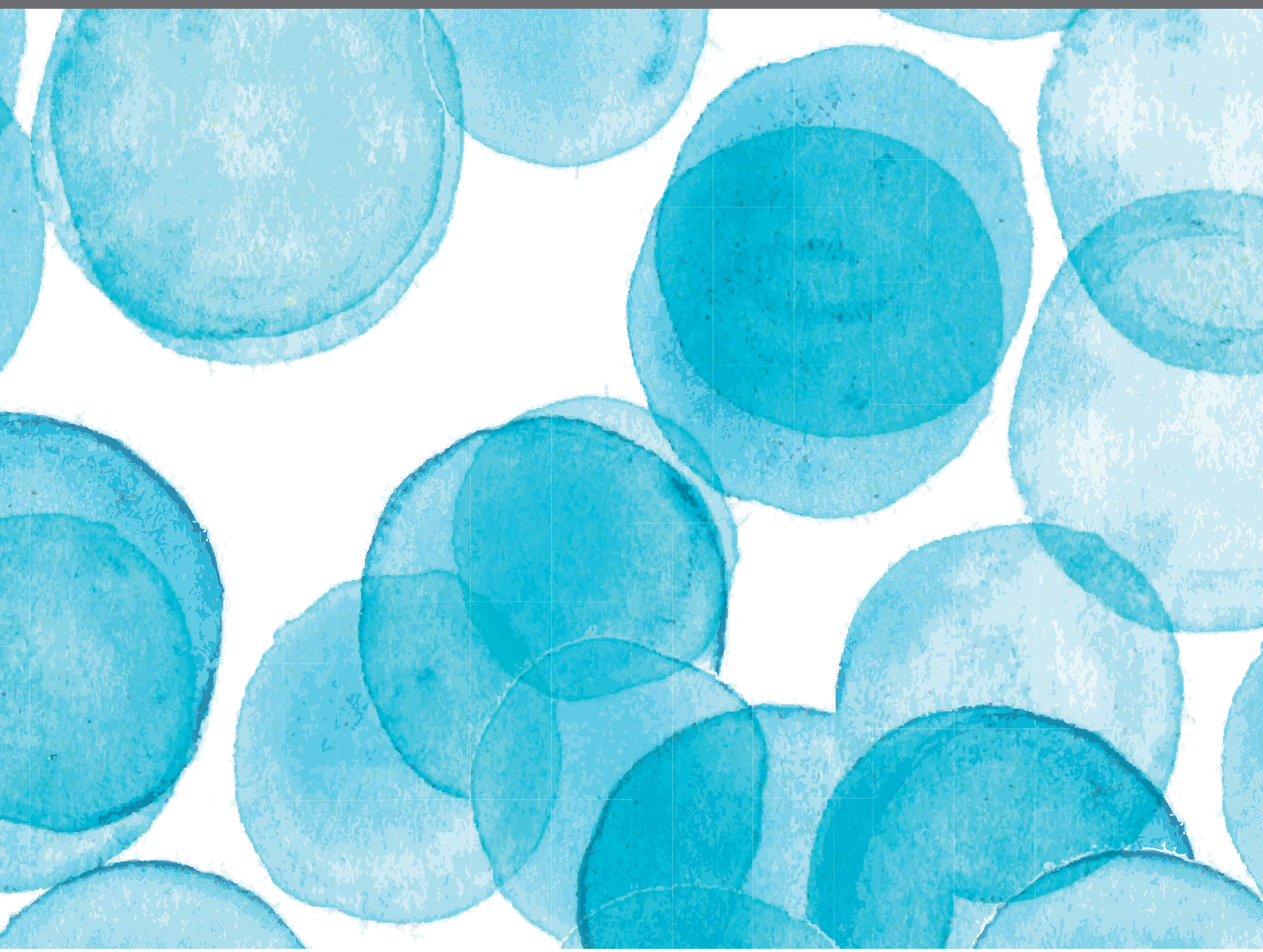

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TOOLS, TECHNIQUES, AND STRATEGIES FOR TEACHING IN A REAL-WORLD CONTEXT WITH MICROBIOLOGY

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EDITORIAL
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Editorial: Research Topic Tools, Techniques, and Strategies for Teaching in a Real-World Context With Microbiology

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Editorial on the Research Topic

Research Topic Tools, Techniques, and Strategies for Teaching in a Real-World Context With Microbiology

The COVID-19 pandemic has emphasized the importance of teaching microbiology in a way that motivates learners to address global challenges and allows participants to practice skills that can be used to harness the power of the microbes around and within us. This themed issue on "Tools, Techniques, and Strategies for Teaching in a Real-World Context with Microbiology" includes twenty-five articles written by authors from around the world that describe innovative approaches for contextualizing microbiology and the impacts of these approaches on student learning (**Figure 1**).

Of these articles, fourteen describe lab-based courses in which learners are engaged in inquiry-based learning or Course-Based (Undergraduate) Research Experiences (CUREs/CREs) (Adkins-Jablonsky et al.; Alvarado et al.; Bueso-Bordils et al.; Furrow et al.; Gordy and Goller; Maicas et al.; Petrie; Stiemsma et al.; Sun et al.; Zelaya et al.; Fuhrmeister et al.; Fuller and Torres Rivera; Greenman et al.; Lo and Le). The learners in these courses range from first-year undergraduates to Ph.D. students, and the institutions at which they are enrolled include research-intensive (R1) universities, primarily undergraduate institutions (PUIs), and minority-serving institutions (MSIs) in multiple countries.

For example, crowdsourced and highly collaborative initiatives such as Tiny Earth offer instructors across the globe frameworks for engaging student researchers in the process of antibiotic discovery and raising awareness of the challenges of emerging antibiotic resistance. In this themed issue, Maicas et al. and Bueso-Bordils et al. describe adaptations of Tiny Earth in Spain. Alvarado et al. offer new improvements to the Tiny Earth curriculum that promise new discoveries and service-learning opportunities for participants, Aho et al. describe a CURE in which learners identified non-pathogenic *Neisseria* strains with antimicrobial activity against *Neisseria gonorrhoeae*, and Fuhrmeister et al. report a novel CURE that complements the focus of Tiny Earth on antibiotic discovery by engaging learners in environmental antimicrobial resistance surveillance. While each of these initiatives focus on the global challenge of antibiotic resistance, they each take a different approach, providing valuable insight and options for instructors interested in implementing a Tiny Earth course or incorporating aspects of antibiotic discovery and/or antimicrobial resistance surveillance into existing lab courses.

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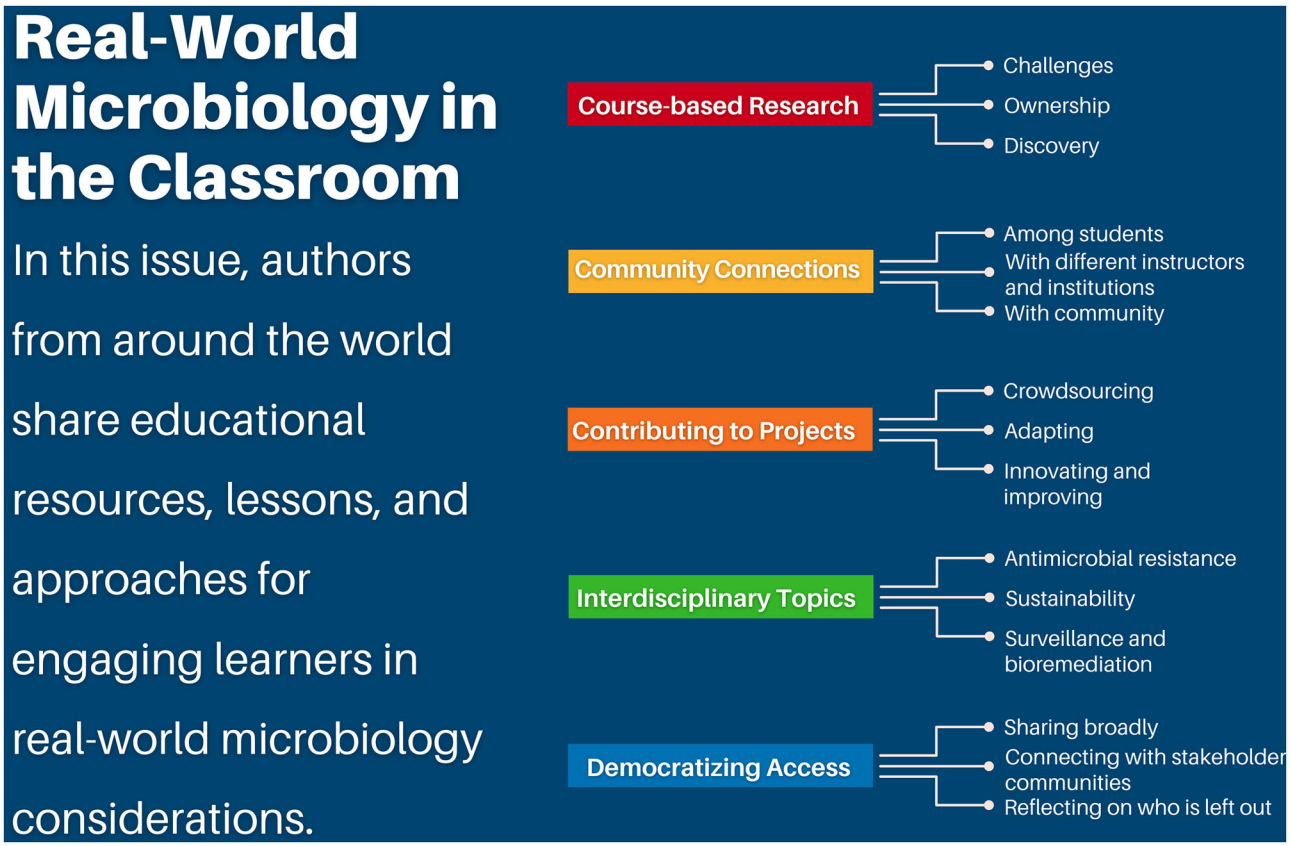

FIGURE 1 | Real-world microbiology in the classroom. In this issue, authors from around the world share educational resources, lessons, and approaches for engaging learners in real-world microbiology considerations. Course-based research experiences challenge learners, foster ownership of projects, and inspire discovery. Community connections can be fostered among students, with different institutions and instructors, and with the community. Participants and educators can contribute to projects through crowdsourcing, adapting resources and projects, and innovating and improving the student experience and research methodologies. Interdisciplinary topics in this issue include antimicrobial resistance, sustainability, and surveillance and bioremediation using microorganisms. With the vision of democratizing access, educators and students can share broadly, connect with stakeholder communities, and reflect on who is left out.

Empowering students to discover the origin of antibiotics and the prevalence of antibiotic resistance prepares new researchers in fundamental wet-lab and bioinformatics skills. Equally important, science communication training equips students to share their findings and research experiences and reflect on the process of scientific discovery. Lloyd describes how blogging activities kindle intrinsic motivation in learners in a medical microbiology course. Linares et al. report a collaborative service learning project in which students and faculty work in interdisciplinary teams to engage marginalized communities in Madrid by developing educational outreach activities using movies related to specific infectious diseases that community members indicate they would like to understand better. Sagmeister et al. describe the use of role playing activities to address antibiotic resistance and societal issues. These articles share useful experience and ways to encourage instructors to make courses more dynamic and connected to the public.

Beyond antibiotic resistance, authors report courses engaging students through the connection of microbiological concepts to multiple other topics with clear impacts on human health. Potter's students contribute to a service-learning project in which they educate their peers on handwashing and vaccination. Fuller and Torres Rivera's students interview family members to learn about traditional home remedies used within their family, and then test the efficacy of these remedies in preventing the growth of various microbes. Both of these innovative approaches allow learners to ground their scientific learning in their own lived experiences, by allowing students to connect that learning to the health habits of their campus community or to their family's traditions and culture.

Another topic with clear real-world relevance and opportunities for students to practice cutting-edge techniques is the microbiome, the genomic potential of microbial communities. The microbiome was once a complex concept that was only mentioned in a few upper-division courses. Now, however, metagenomics has been democratized through a series of educational initiatives such as the Bean Beetle Microbiome Project (Zelaya et al.). Through careful assessment of the impact of exposing students to inquiry-based learning of microbiome communities, this and other projects have highlighted the

opportunities to engage students in the creation and analysis of microbiome datasets. The potential of microbiome-focused CUREs for lower-level undergraduate courses is further demonstrated by both Lo and Le and Stiemsma et al. Lo and Le report that a novel soil microbiome CURE conducted in large-enrollment introductory biology courses resulted in increased research skills as well as self-efficacy, while Stiemsma et al. report that a first-year Students as Scholars course in which learners investigated the relationship between soil salinity in coastal watershed regions and coliform contamination of water resulted in increased confidence and persistence in STEM. In addition to these descriptions of novel microbiome-focused CUREs, Muth and Caplan review educational microbiome resources that have been published. Available resources now include lessons, activities, and entire courses that can be adapted for a variety of different contexts. To increase relevance, established protocols can now be adapted to specific environments or samples that will resonate with students.

Learners in many of these microbiome-focused CUREs gain hands-on experience in environmental sampling, Next Generation Sequencing technologies, and analysis of data sets with environmental relevance. Several other articles in this issue engage learners in connecting microbiology to sustainability and environmental justice through lab-based research and classroom activities. Adkins-Jablonsky et al. report outcomes from three CUREs focused on heavy metal pollution at three different institutions. Gordy and Goller describe a CRE in which undergraduate and graduate learners collaborate to create products sustainably in yeast and explore the social, environmental, and ethical implications of metabolic engineering strategies used to produce foods, medicines, and fuels in yeast through case studies. Shay et al. share a problem-based classroom activity that brings together biochemical and microbiological concepts through which learners address the roles of microbes in degrading paper waste. The scientific concepts addressed in these three articles differ; yet learners in all of these courses gain an understanding of how microorganisms function in the environment and can be used to remediate environmental damage or create products in a sustainable manner.

Another emerging theme in the articles in this issue—both those engaging learners in microbiome research and others—is food microbiology. Petrie's students sequence natural CRISPR arrays in lactic acid bacteria in yogurt, while Gordy and Goller's students produce beta carotene in yeast and learn about the technology used to produce the Impossible Burger through a case study-based approach. Greenman et al.'s students focus on foodborne pathogens by isolating and characterizing environmental *Salmonella enterica* from stream sediments and poultry litter.

Sharing instructional materials and the results of educational studies through this issue and other publications helps build lesson plans and curricula for instructors to adapt and improve. Sharing protocols (e.g., Tiny Earth) (Bueso-Bordils et al.; Maicas et al.) and datasets (Zelaya et al.) lowers the barriers for more institutions to implement similar authentic research activities. Evaluating the impact of tools, techniques, and strategies deployed at different institutions will enrich our understanding of pedagogical choices, student engagement, and instructor needs while increasing student research output.

Beyond instructors sharing their educational materials, Sun et al. describe how they empower students to share their work in an open access online undergraduate research journal that increases access to research while providing fundamental skills to participants, such as scientific writing and peer review. In this way, students can also drive sharing and encourage others to use and build on their findings.

As we consider the rich learning experiences described in the articles in this themed issue as well as those reported in other venues, it is always important to ask, who is being left out? Are there barriers preventing the widespread adoption of these initiatives? Are marginalized populations unable to take full advantage of these resources? If so, how do we include real-world microbiology context in their educational experiences?

Several articles in this issue address some of the barriers that prevent learners and instructors at many institutions from benefiting from the rich educational experiences and activities reported throughout the issue. For example, Nguyen et al. describe a laboratory method that can be used to identify microorganisms in teaching environments that lack access to PCR, and Faist et al. describe the biocrust model system, an accessible and portable ecosystem that can be used to teach concepts ranging from microbial evolution and ecology to structure and function in a range of classroom settings and types of institutions. Smith-Keiling, along with Aparna et al., directly discuss barriers that prevent adoption of inquiry-based labs and CUREs/CREs at resource-limited institutions. Smith-Keiling addresses biosafety challenges that are common in many teaching labs, while Aparna et al. describe an approach for introducing real-world microbiology techniques in environments where learners are typically not exposed to these skills. These innovative approaches will each help to make real-world microbiology classroom experiences available to more students.

Beyond institutional resource limitations that impact instructors' ability to implement CUREs/CREs, we must begin to consider structural and systemic barriers that disparately impact the access of certain groups of students to real-world microbiology experience. The classroom—be it the lecture hall, the lab, or the online space—is not the only option for contextualizing real-world microbiology practices. Professional experiences such as internships and research opportunities for credit are transformative experiences for participants. Unfortunately, these opportunities are not available to all students due to eligibility requirements, funding, and institutional limitations. For example, many opportunities for independent undergraduate research experiences funded by US federal funding agencies exclude students who are undocumented or DACA recipients. Other students meet federal eligibility rules but not the selection criteria set by individual research experience for undergraduates (REU) programs. In particular, students who have not followed a traditional path, who may have had a rocky start to their undergraduate career but who show passion and promise, will not rise to the top of the candidate pool when criteria include GPA and other traditional metrics.

To create undergraduate research experiences that are open and accessible to all students, educators and institutions must think outside the usual framework and funding sources. Those interested in developing open and accessible research opportunities can draw on the virtual tools and connections developed out of pandemic-driven necessity to create experiences that blur institutional boundaries, allowing learners and mentors to interact and collaborate regardless of location. Open science and open educational practices can allow for more access to the guides that instructors can then use to mentor students both in person and virtually.

While creating virtual research experiences is less expensive than residential REUs, funding for student stipends is critical: unpaid research internships serve to consolidate valuable research experiences among those who can afford to work for free. Thus, PIs must seek out agencies and foundations that are able to fund student stipends regardless of factors such as immigration status. For example, Code for Science and Society's Virtual Event Fund (Virtual Event Grants, 2020) has provided funding for MORE (Mentored, Open Research Experiences), a pilot online undergraduate research experience through which students receive training in open data science practices focused on microbiome analysis and other genomic research skills, carry out mentored research projects, and receive a stipend. This approach of creating open, online resources to train learners in skills necessary for real-world microbiology research can be used not only to support cross-institutional undergraduate research programs, but also to further disseminate the innovative courses and activities published in this issue and to increase the number of learners who can benefit from them.

As you review the articles in this issue for topics of interest and initiatives you can connect with or incorporate, we encourage you to reflect on how you can expand their reach—not only by implementing them at your institution, but by thinking about how you can begin to dismantle the barriers that prevent students from enrolling in your courses, studying microbiology at your institution, or undertaking independent research. After all, sharing these educational experiences and resources benefits current students and future researchers, improving education and promoting discovery.

AUTHOR CONTRIBUTIONS

CCG and CLG wrote and edited the text. MR and MV conceived the open mentoring program and edited the text. All authors contributed to the article and approved the submitted version.

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REFERENCES

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METHODS
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The Bean Beetle Microbiome Project: A Course-Based Undergraduate Research Experience in Microbiology

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Course-based undergraduate research experiences (CUREs) are an effective means of transforming the learning and teaching of science by involving students in the scientific process. The potential importance of the microbiome in shaping both environmental health and disease makes investigations of microbiomes an excellent teaching tool for undergraduate microbiology. Here, we present a CURE based on the microbiome of the bean beetle (*Callosobruchus maculatus*), a model system for undergraduate laboratory education. Despite the extensive research literature on bean beetles, little is known about their microbiome, making them an ideal system for a discovery-based CURE. In the CURE, students acquire microbiological technical skills by characterizing both culturable and unculturable members of the beetle gut-microbial community. Students plate beetle gut homogenates on different media, describe the colonies that are formed to estimate taxonomic diversity, extract DNA from colonies of interest, PCR amplify the16S rRNA gene for Sanger sequencing, and use the NCBI-nBLAST database to taxonomically classify sequences. Additionally, students extract total DNA from beetle gut homogenates for high-throughput paired-end sequencing and perform bioinformatic and statistical analyses of bacterial communities using a combination of open-access data processing software. Each activity allows students to engage with studies of microbiomes in a real-world context, to apply concepts and laboratory techniques to investigate either student or faculty-driven research questions, and to gain valuable experiences working with large high-throughput datasets. The CURE is designed such that it can be implemented over either 6-weeks (half semester) or 12-weeks (full semester), allowing for flexibility within the curriculum. Furthermore, student-generated data from the CURE (including bacterial colony phenotypic data, full-length 16S rRNA gene sequences from cultured isolates, and bacterial community sequences from gut homogenates) has been compiled in a continuously curated open-access database on the Bean Beetle Microbiome Project website, facilitating the generation of broader research questions across laboratory classrooms.

Keywords: scientific teaching, undergraduate education, course-based undergraduate research, insect microbiomes, 16S rRNA gene sequencing, microbial community analysis

INTRODUCTION

In recent years, course-based undergraduate research experiences (CUREs) have gained widespread attention as being effective alternatives to "cookbook" style teaching approaches in science, technology, engineering and mathematics (STEM) (Corwin Auchincloss et al., 2014). CUREs allow students to actively participate in the scientific process, experience discovery, do broadly relevant work, practice collaboration, and build on their growing knowledge and experience via iteration (Corwin Auchincloss et al., 2014). By modifying the formal curriculum to integrate an authentic research experience into the classroom, CUREs are able to offer a greater number of students the educational benefits of traditional research experiences (i.e., internships or apprenticeships in faculty research labs) (National Academies of Sciences, Engineering, and Medicine, 2017). These benefits include greater scientific self-efficacy, improved critical thinking skills, increased grades, increased interest in science careers, and higher graduation and retention rates (Lopatto, 2004; Seymour et al., 2004; Corwin et al., 2015a; Staub et al., 2016).

Interdisciplinary research on microbiomes, or the collection of microorganisms and their genes within a given environment, has uncovered the vast diversity and complexity of the microbial world. Microorganisms and their activities are essential for maintaining both human and environmental health (Timmis et al., 2019). In both plants and animals (including humans), microbes assist in the breakdown and absorption of essential nutrients and minerals (Nicholson et al., 2005; van der Heijden et al., 2008) and the development of immunity (Bäckhed et al., 2012). Environmental microbes residing in soil and water recycle nutrients on a global scale (Chapelle, 2000), degrade and transform toxins (Anderson and Lovley, 1997), and contribute toward the removal of greenhouse gases from the atmosphere (Mitchell et al., 2010). Given their broad relevance in shaping the health of our world, microbiomes provide an excellent and engaging topic for undergraduate students and ample opportunities for discovery-based research (Wang, 2017).

Despite the educational potential for laboratory classrooms, microbiome research can be challenging. Many biological systems are highly complex, involving a diverse array of microbes with confounding variation both within and between populations (Spor et al., 2011; Linnenbrink et al., 2013; Goodrich et al., 2014). Disentangling the many genetic and environmental factors shaping this variation is challenging, particularly in organisms that are not easy to control experimentally. Many insect species have served, and continue to serve, as tractable systems for studying the potential factors that shape host-microbe associations (Engel and Moran, 2013; Douglas, 2019). Insect microbiomes tend to be simpler than their vertebrate microbiome counterparts (Wong et al., 2011; Hammer et al., 2017). Additionally, insects can be reared under controlled, laboratory conditions in large numbers, facilitating precise manipulation of environmental factors that may influence microbial associations.

Bean beetles (*Callosobruchus maculatus*) are a tractable insect model system that has been used widely in inquiry-based laboratory education. Bean beetles are inexpensive, commercially available, reproduce rapidly and in large numbers, develop quickly, and are easy to rear and maintain in a classroom environment (Beck and Blumer, 2007). Bean beetles also have broad global relevance, as they are a stored-product pest of dried beans and cause significant economic damage worldwide (Tuda et al., 2006). Although bean beetles have been extensively studied in a wide range of biological disciplines (Beck and Blumer, 2007), very little is known about their microbiome, making them an ideal system for a discovery-based CURE in microbiology. Bean beetles are easily amenable to microbiome experimentation, allowing student researchers to manipulate factors that might affect gut bacteria in ways that would not be possible with vertebrates or other insect model systems.

The Bean Beetle Microbiome Project is a large-scale, multi-year STEM-Education research collaboration with the overarching goal of understanding the role of student autonomy in using scientific practices in a discovery CURE for students across diverse institutions. Here, we present and describe the course materials developed for the Bean Beetle Microbiome CURE that can serve as an inquiry-based course curriculum for undergraduate microbiology laboratories. We also present preliminary survey data from upper-level undergraduates at three different institutions who participated in the first set of implementations of the BBMP-CURE during the Fall 2019 academic term. In the BBMP-CURE, students integrate microbiological, molecular, and bioinformatic techniques to characterize both culturable and unculturable members of the beetle gut-microbial community. We discuss how the CURE can be implemented in either 6-week (half semester) or 12-week (full semester) versions, allowing flexibility within the curriculum. The 6-week format can also be implemented by instructors within institutions that follow the quarter-system. Depending on the research interests of the faculty, many aspects of the CURE outlined below can be easily modified such that students and instructors can take greater ownership of the research questions asked, methods used, and overall research experience. Student-generated data may contribute to ongoing faculty research that subsequently leads to publications. Additionally, faculty have the option of sharing their classroom data within a curated open-access database on the Bean Beetle Microbiome Project website, facilitating the generation of broader research questions across laboratory classrooms and institutions.

MATERIALS AND EQUIPMENT

Learning Objectives

The activities below are designed for students to expand on laboratory skills that they may have practiced in previous introductory laboratory courses (e.g., pipetting, culturing bacteria, and record keeping). However, activities can be easily amended for an introductory class such that basic laboratory skills can be introduced at the time of or prior to performing the activity.

The overall learning objectives for the CURE are for students to be able to:

(1) Describe the impact of microbes on our living planet and their host environments.
(2) Sample and compare microbial communities (microbiomes).
(3) Formulate testable hypotheses to address a research question and design an experiment to test the hypotheses.
(4) Identify and apply the microbiological, molecular, and bioinformatic techniques used to study microbiome data.
(5) Analyze 16S rRNA gene sequence data with common techniques used in microbiome research.
(6) Interpret figures generated from microbiome sequence data.
(7) Communicate findings to peers via oral or poster presentations and scientific writing (report).

To facilitate project ownership, an additional learning objective may be for students to "Discuss and pose a meaningful research question that builds on prior research." Learning objectives can be modified and amended by the faculty based on the specific research questions asked. Published example rubrics for evaluating student presentations are available (e.g., Kishbaugh et al., 2012).

Beetle Rearing and Maintenance

A living culture of bean beetles may be purchased from Carolina Biological Supply Company (Burlington, NC, United States, item number 144180) or Ward's Science (Rochester, NY, United States, item number 470163-616). Beetles are easily maintained on a countertop in small jars or containers with dried beans (**Figure 1**). Bean beetles prefer and grow best on black-eyed peas (*Vigna unguiculata unguiculata*); however, beetles grow well on at least eight different bean hosts (see Laboratory Methods at beanbeetles.org), and beans chosen can vary based on the experimental questions to be addressed. Female beetles glue their eggs to the surface of beans. Approximately 8–10 days after oviposition, the beetle larva "hatches" from the egg by burrowing from the egg into the bean, where the developing larva feeds off the bean endosperm. When stored at 25–30°C and developed on black-eyed peas, an adult beetle emerges from the host bean after approximately 25–35 days and becomes sexually mature 24–36 h after emergence. Larval development times may vary depending on host-bean species and environmental conditions (e.g., incubating temperature and humidity). Adult beetles do not eat or drink liquid water, and adults live for approximately 10–14 days. Currently, no U.S. federally mandated permits or requirements are needed to house and use bean beetles for educational or research purposes. Individual U.S. State Department of Agriculture requirements remain in force. The authors advise that educators verify with their home institutions to ensure that no local or institutional requirements exist.

While rearing beetles for classroom activities, it is important to plan accordingly to synchronize emergence times with experiments. Beetle cultures should be grown for a few months in consistent laboratory conditions on the same bean species so as to accurately predict emergence times. Additionally, maintaining replicate lines (on the same bean-host) that have alternating emergence periods can ensure a consistent supply of adult beetles. More details on working with bean beetles can be found at https://www.beanbeetles.org/handbook/.

Equipment, Supplies, and Reagents

A current list of all equipment, supplies, and reagents used in the different modules of this CURE can be downloaded at https://www.beanbeetles.org/new_website/wp-content/uploads/2019/08/Combined-Equipment-and-Supplies-List-1.pdf. The CURE is designed to use common laboratory equipment available in general microbiology and molecular biology laboratories.

For the bioinformatic analysis portion of the CURE, we designed the activities to utilize free, web-based software that does not require advanced computer science skills (e.g., command-line usage or coding skills). Access to a computer lab would facilitate the implementation of the bioinformatical analysis activities; however, students also may use their personal laptop computers while accessing a university internet connection. Alternatively, bioinformatic analysis may be designated as outside laboratory assignments, allowing students more flexibility to access the technology (computer, internet-connection) necessary to complete the analysis. For example, in Spring 2020, we conducted bioinformatic analyses with undergraduate students in a completely on-line distance learning format.

For taxonomic identification of isolated colonies from beetle gut-homogenates, The Basic Local Alignment Search Tool (BLAST)[1] is used to identify closest bacterial relatives. For whole community microbiome analysis, spreadsheets containing metadata are created in either Microsoft Excel (Office 365, 2019) or Google Sheets. Bioinformatic analyses are conducted using DNA Subway, which was developed by the DNA Learning Center at Cold Spring Harbor Laboratory. It is a streamlined, classroom-friendly version of the popular QIIME2 microbiome bioinformatics software (Bolyen et al., 2019) and is available via CyVerse[2], a free-to-use cyber-infrastructure funded by the National Science Foundation that provides computational resources to researchers and educators nationwide (Goff et al., 2011; Merchant et al., 2016). Detailed protocols for all bioinformatics activities are available at https://www.beanbeetles and https://org/microbiome/resources-for-online-teaching/.

DNA Sequencing

The lessons presented here make use of both Sanger and Next-Generation Sequencing technology in order to provide students a greater breadth of understanding of the tools available to study both cultivable and non-cultivable bacteria at the genetic level (single colony/species vs. whole communities). Currently, the cost for 16S V4 Miseq amplicon sequencing ranges from $55 to $85/sample depending on the number of samples in a single run and whether you use a commercial or academic institution sequencing facility. A meaningful microbiome experiment will

[1] http://blast.ncbi.nlm.nih.gov/
[2] https://www.cyverse.org

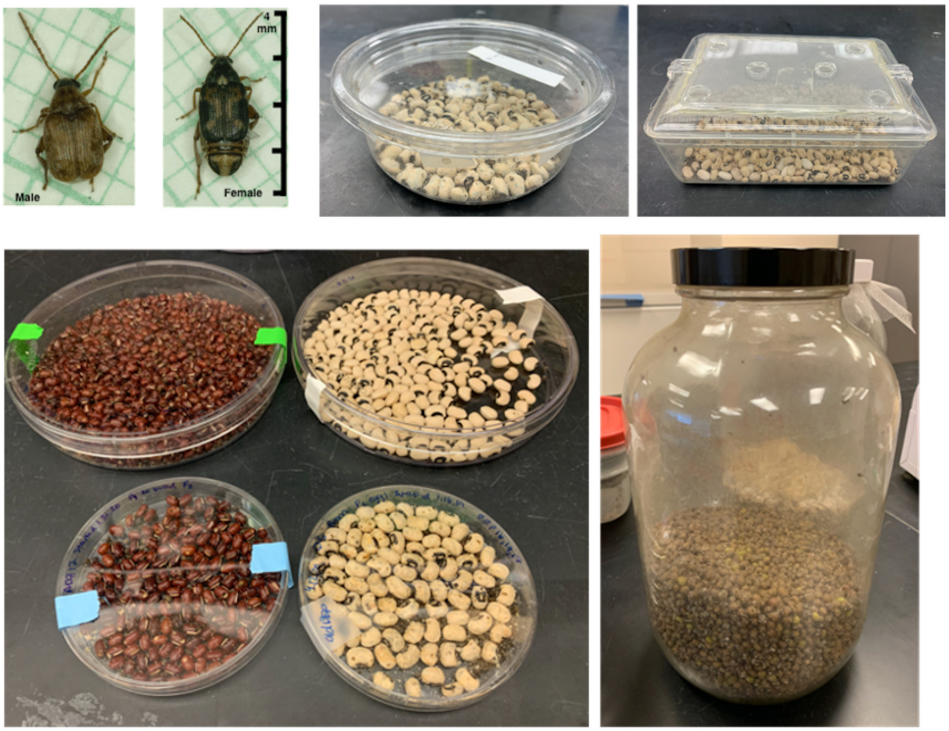

FIGURE 1 | Maintaining bean beetles in the laboratory classroom. Adult (**A**-male, **B**-female) beetles range from about 2–4 mm in length. Beetles cultures can be maintained in a variety of containers filled with dried beans. **(C)** Small round plastic container with lid. **(D)** Plastic bin with lid, holes have been drilled into the lid and lined with filter mesh to allow for gas circulation. **(E)** Petri dishes of various sizes filled with either adzuki beans or black-eyed peas. **(F)** A large glass jar filled with mung beans.

likely require a minimum of five samples in each of two treatments, or a total of 10 samples, so whole community sequencing will cost between $550 and $850/class. The cost of NGS-technology can be prohibitive (Wang, 2017). Colleges and universities that cannot secure funding for 16S whole community sequencing may wish to use sample data sets available at https://www.beanbeetles.org/microbiome/resources-for-online-teaching/. As this CURE also incorporates Sanger sequencing of cultivable bacteria, instructors have the option of performing a proxy for the whole community analysis (from NGS technology) with a class-wide Sanger sequencing dataset as a lower-cost alternative (see Student handout on community analysis of colony-based sequence data for this alternate activity).

Classroom Size and Considerations

The CURE outline below was designed for a laboratory course meeting once a week for a minimum of 3 h. The outlined materials and procedures can accommodate a laboratory of 24 students. Activities were designed for students to work as collaborative teams (e.g., 6 groups of 4 students each). Notably, while the BBMP-CURE was originally designed for hands-on laboratory instruction, much of the components, particularly the modules relating to sequence and community analysis, can be performed in fully virtual-classroom formats.

Student and Instructor Handouts

All handouts for each laboratory session can be found at https://www.beanbeetles.org/workshops/curriculum-materials/. Instructor handouts provide additional information for preparation of materials. Student handouts provide step-by-step protocols for activities to be carried out for each lesson.

METHODS

The BBMP-CURE has the flexibility of being carried out in either full or half-semester implementations. Both full- and half-semester timelines can be found in **Supplementary Tables S1,S2**, respectively. The timelines also contain a list of handouts and instructional materials related to weekly activities. These materials can also be found at https://www.beanbeetles.org/workshops/curriculum-materials/. Timelines are constructed assuming a laboratory course that meets once a week for at least 3 h. All protocols and sample datasets can be downloaded at https://www.beanbeetles.org/microbiome/resources-for-online-teaching/.

Full-Semester Implementations

The first class period serves as an introduction to microbiome research in general. The introduction includes a discussion of how insect microbiomes can be used as research models. An

introduction into bean beetles as the model organism provides information on bean beetle ecology, sex-based identification, life-cycle, and agricultural impact. Additionally, students are asked to read published studies relating to bean beetles and microbiome research in various biological systems. Background reading can be assigned as either as a pre-lab or in-class assignments (see suggested reading materials).

The introductory lecture and reading assignments provides the background information and context to facilitate the development of a research question, hypothesis, and experimental design, including identification of control versus experimental groups. The BBMP-CURE uses a guided-inquiry approach to guide students through these initial stages of the project. The research question to be studied is either provided by the instructor (faculty-driven research question), or it is generated by the students themselves through an iterative process of discussion and feedback. Regardless of how the research question is generated (either by the students or by the instructor), students are expected to predict the outcome of the study (generate a hypothesis), as well as to design and conduct the experiment to test their hypothesis, including identifying the control versus experimental groups. Instructors may choose to structure a more faculty-driven or student-driven approach. The approach taken may depend on student level (e.g., freshman versus senior), course type (e.g., introductory vs. advanced courses), or course objectives. Additionally, the instructor may choose to have students formulate their research question(s) based on class consensus, or alternatively, if the budget permits, different groups may choose to answer different questions. Example research questions that have been generated by students of the BBMP-CURE using a guided-inquiry approach can be found in **Supplementary Table S3**.

By week 2, students and instructors have agreed upon the research question(s) to be investigated. Subsequently, students extract DNA from beetle gut homogenates for whole-community sequencing. The timing of DNA extraction depends on the nature of the experiment being conducted. Comparisons of microbiomes from individuals taken from mass cultures (for example, comparing the microbiomes of males and female beetles) may be performed as early as week 2 or 3 if those cultures were previously established. However, a manipulation experiment will require three weeks or longer for the development and emergence of adults (for example, the effect of a host shift on the microbiome community). In classroom settings, waiting for adult emergence is necessary, as larvae are embedded in beans and may be hard for students to successfully isolate without contamination. The steps outlined below describe DNA extraction using the Qiagen DNeasy Blood and Tissue Kit. Qiagen DNeasy Blood and Tissue kit was chosen for this module based on previous literature that demonstrates its successful retrieval of insect-microbiome DNA (Furgeson et al., 2018).

DNA is extracted using a modified protocol for the purification of total DNA from insects (see both Instructor and Student Handouts on DNA extraction for the full semester implementation[3]). Prior to extraction, live adult beetles must first be transferred to a small sterile container (e.g., Petri-dish or microcentrifuge tube) and sacrificed by freezing (−20°C) for a brief period of time (e.g., 3-min). Students can perform the freezing step, or alternatively instructors may sacrifice the beetles before the start of the class session. Once students receive their sacrificed beetles, they should record relevant specimen information (e.g., host diet, sex, age, etc.) prior to surface sterilization. Then, surface-sterilized beetles are transferred to a 1.5 mL microcentrifuge tube containing 180 uL of Buffer ATL. Using a sterile disposable pestle, beetles are crushed to release microbial cells from the beetle gut. The extracted DNA can be quantified (e.g., with a Nanodrop), and stored at −20°C until sequencing.

It is not uncommon for students to extract insufficient DNA on their first attempt. Therefore, week 3 of the full-semester implementation has been designated as an iteration day, allowing students the opportunity for a second attempt at DNA extraction. Students can repeat unsuccessful extractions, or students who successfully extracted sufficient DNA in week 2 can use the time to isolate DNA from an individual from another treatment group to increase sample size. Alternatively students who do not need to repeat an extraction can proceed to culture bacteria from gut homogenates (see below).

The DNA extraction step is ideally performed early in the semester (week 3) in order to provide ample time for sequencing to be performed. Although typical turnaround times for paired-end whole community sequencing can vary, we have allotted ∼4 weeks from the time the DNA is sent out to retrieval of sequence data for community analysis.

Once DNA extractions have been performed, students initiate culture-based analysis of microbes isolated from beetle gut-homogenates. Beetles are surface sterilized and transferred to a 1.5 mL microcentrifuge tube containing 500 uL of 0.9% sterile saline solution. Each beetle is crushed with a sterile pestle and cell-homogenates are serially diluted and plated on different solid-media to obtain bacterial cultures from the beetle microbiome. The current module outlines the use of a general growth media (nutrient agar) as well as the use of selective media for Gram-positive (phenyl-ethyl alcohol) and Gram-negative (eosin-methylene blue) bacteria. Students allow their plates to grow at room temperature (25°C) until the following class period. However, the instructors may opt to utilize any number of general or selective medias to appropriately pursue the specific research question being addressed by their students. Additionally, initial growth in liquid cultures can be used to pursue questions related to physiological or metabolic capabilities of the beetle microbiome.

The following class period (week 4), students check the growth of their microbial cultures. They observe and record the phenotypic characteristics of grown colonies. Using a colony-based PCR approach, students then amplify DNA from specific colonies of interest for taxonomic identification via Sanger sequencing of the 16S rRNA gene (see both Instructor and Student Handouts on colony-based PCR). Here it is important to save students' plates, as the following week will allow for iteration (a second attempt at PCR for any groups who do not obtain a successful amplified

[3]https://www.beanbeetles.org/workshops/curriculum-materials/

product). Additionally, students who took advantage of the iteration for DNA extraction in week 3 may catch-up this week by crushing beetles and plating the gut-homogenates onto solid-media.

In week 5, students who performed colony-based PCR in week 4 visualize their amplified products using gel electrophoresis. Additionally, week 5 can serve as an iteration day for students who may still need to catch up (either they need to perform colony-based PCR on their isolates for the first time, or their PCRs did not yield enough DNA and they need to redo their PCR). All successfully amplified DNA products should then be sent out for Sanger sequencing.

As Sanger-sequencing turn around is typically fast (∼24–48 h), students should have their Sanger-sequence data by the following class period (see Student Handout on BLAST analysis of sequencing data). Students use the NCBI-nBLAST database to taxonomically classify the colony-based PCR sequences, and create a taxonomy table of the bacterial genera identified in the microbiomes of the beetles in their experiment. This class session serves as an introduction to bioinformatics tools and computational software to compare genetic sequences for taxonomic relatedness. The concepts learned in these sessions will continue to be built upon as they proceed through the semester.

In week 7, students are introduced to community analysis. They are first introduced to this concept by performing community analysis on the phenotypic data that they previously collected on microbes isolated on solid-media. They connect the phenotypic data from week 4 with the colony-based PCR sequence data from Sanger sequencing. They calculate alpha-diversity statistics based on the taxonomy table of different taxa identified from the colony-based PCR sequence data and determine similarities and differences in diversity between samples (Blumer and Beck, 2020). The worksheet for these activities can be downloaded in the course materials for week 7 of the full semester.

By week 8, the whole community (paired-end) sequence data should be available. These data permit students to expand on the community analysis by identifying bacterial taxa in the whole-community dataset using DNA Subway. Weeks 9 through 13 are then reserved for bioinformatic analysis of microbial community sequence data. Since this is the most challenging technical aspect of the CURE, several sessions are allotted to complete this activity. Multiple options are available here to cater to varying degrees of experience and pre-requisite knowledge of students and instructor. These include community analysis using the R Statistical Package, spreadsheets in Excel or Google Sheets, or the free web-based Shiny App ranacapa (Kandlikar et al., 2018). Alternatively, the semester can be modified such that activities are assigned as outside classwork (homework), allowing for greater flexibility of the curriculum.

In the final week, students develop their scientific communication skills by presenting their results. Instructors may choose presentation methods to match their course learning objectives (scientific abstract, full scientific report, seminar style presentation, poster presentation). Undergraduates may also take advantage of opportunities to present research outside of the classroom at their home institutions (for example, a departmental undergraduate research symposium or college-wide science symposium).

Half-Semester Implementations

Similar to the full-semester implementation, the first class period for the half-semester implementation serves as an introduction to insect microbiomes in general and bean beetles as the model organism. Additionally, the research question and experimental design should be discussed and agreed upon during the first class session. However, the introduction period is shortened such that students initiate their research project in the first class period. Once students and instructors agreed on the research question(s) to be investigated, students are provided with their specimen beetles and follow surface sterilization and homogenization steps to plate beetle-gut homogenates onto general and selective media.

The following class period (Week 2), students check the growth of their microbial cultures, observe and record the morphological characteristics of grown colonies, and perform colony-based PCR on their isolated colonies for taxonomic identification via Sanger sequencing (see both Instructor and Student Handouts on colony-based PCR for the half-semester implementation[3]). Again, all plates can be saved for the following week in case students require an iteration day. Finally, students obtain a fresh collection of beetles to perform total DNA extraction for whole-community sequencing using the Qiagen DNeasy Blood and Tissue Kit. Completing DNA extraction may require students to perform laboratory work outside of scheduled class time, or for someone to perform these extractions after students initiate the process by grinding a beetle in Buffer ATL (see both Instructor and Student Handouts on DNA extraction for the half-semester implementation[3]).

In week 3, students perform gel-electrophoresis on their amplified DNA (see Instructor handouts on electrophoresis). Typically, electrophoresis can be completed in about 1 h, at which point any groups that did not successfully amplify DNA may elect to re-do their PCR on a picked colony. Then, successfully amplified DNA products may be sent out for Sanger sequencing. In a typical 3-h laboratory course, students would have enough time to run a gel and re-do PCR for any unsuccessful amplifications. However, running a gel on the second PCR attempt to confirm amplification may require students to perform laboratory work outside of scheduled class time.

The following class periods (weeks 4, 5, and 6), students begin the bioinformatic analyses. They use the NCBI-nBLAST database to taxonomically classify sequences (see Student handout on BLAST analysis of sequencing data), perform community analysis on phenotype and colony-based PCR sequence data (see student handout on community analysis of phenotype data), and perform community analysis of their whole microbiome sequencing data (see handouts corresponding to community analysis of sequencing data for week 6 of the half-semester implementation). The half-semester implementation allows insufficient time for students to learn to use DNA Subway

to create a taxonomy table for subsequent community ecology analysis. Therefore, it is necessary for the instructor or teaching assistant to perform the data processing in DNA Subway and present students with the resulting taxonomy table for analysis. As in the full-semester implementations, students can present their results in the final week of the project in either the written or oral formats suggested above.

ANTICIPATED RESULTS AND DISCUSSION

Timmis et al. (2019) recently argued that microbiology literacy is one of the most important skills society will need to solve 21st century problems. Before long, novel therapies on microbiome technology will become widely available (Surana, 2019). However, misconceptions and misinformation persists (Ma et al., 2018). Undergraduate university courses represent an excellent opportunity to introduce students of microbiology to the exciting and topically relevant field of microbiome research, as they represent the future leaders and global citizens that will be best equipped to dispel misconceptions and raise microbial literacy among the general public.

While still in its early stages, microbiome research has grown rapidly and garnered the attention of both scientists and non-scientists alike, providing an excellent and engaging topic area for undergraduate research experiences. Inquiry-based activities for student microbiome research have previously been developed for aquatic ecosystems (Boomer et al., 2002; Gibbens et al., 2015; Agate et al., 2016), soil (Martinez-Vaz et al., 2015; Finer et al., 2016; Rahman et al., 2016), public spaces (Muth and McEntee, 2014; Weber and Werth, 2015), and the human microbiome (Wang et al., 2015; Debelius et al., 2016; Garbarino and Mason, 2016; Lentz et al., 2017; Weber et al., 2018). Here we present a CURE using the bean beetle (*Callosobruchus maculatus*) as a model animal system for undergraduate microbiology laboratory courses.

The Bean Beetle Microbiome CURE is aimed to provide students with an authentic research experience as part of the course curriculum that enables them to develop skills related to the scientific process. Studies have shown that research experiences provide students with a greater sense of autonomy and ownership of their projects (Lopatto, 2003; Hanauer et al., 2012), which also leads to additional educational benefits, such as increased intention to pursue science careers. When research experiences are incorporated as part of the course curriculum, there are different inquiry-based approaches that can be used, each with its own set of expectations for both instructor and student (D'Avanzo, 1996; Colburn, 2000; Buck et al., 2008; Weaver et al., 2008). Faculty who would like to implement the CURE presented here in their laboratory classrooms may choose the degree of student involvement for deciding the research question and what inquiry-based approach to use. The novelty and relevance of the question addressed may depend on several factors, including the educational level of the student (e.g., introductory vs. advanced) as well as the level to which the research design is guided by faculty.

An important component of our current research on the BBMP-CURE is the level of student autonomy, or the level of responsibility, related to the development of the research question addressed. Our approach provides two levels of autonomy that we categorize as either low-autonomy (faculty-driven questions) or high-autonomy (student-driven questions). While we hypothesize that a greater level of student autonomy will have a positive effect on student outcomes, it is anticipated that students will strengthen their science-process skills regardless of the level of autonomy chosen, as they analyze the results of their experiments, draw conclusions, connect their research to the broader literature, and communicate their results. Our ongoing research based on assessments of survey data for past and future implementations of the BBMP-CURE will be used to test this hypothesis.

In the fall and spring semesters of the 2019–2020 academic year, a total of 10 faculty participants from six different institutions (most of which were minority-serving institutions) implemented the BBMP-CURE (**Supplementary Figure S1A**). Due to the interdisciplinary nature of the CURE, it was successfully implemented in undergraduate laboratories of various life-science disciplines and student class-levels (**Supplementary Figures S1B,C**). Students who participated in the BBMP-CURE were asked to respond to questions from the Persistence in the Sciences Survey (PITS) (Hanauer et al., 2016) and the Laboratory Course Assessment Survey (LCAS) (Corwin et al., 2015b). As our study did not include a "traditional lab" control, we included two previously published studies that used these same instruments to assess inquiry-based versus traditional laboratory instruction as benchmarks for comparison (Corwin et al., 2015b; Hanauer et al., 2017). Our preliminary student survey data on the LCAS (**Supplementary Table S4**) shows that students rated the BBMP-CURE highly on the Discovery and Relevance scale (28.2 ± 6.3 Full-Semester/Low Autonomy; 28.88 ± 1.81 Full-Semester/High Autonomy; 23.5 ± 11.83 Half-Semester/Low Autonomy), suggesting that students perceived that the activities they performed could lead to discovery of something new and were of interest to the scientific community. Additionally, results of the PITS survey (**Supplementary Table S5**) showed students scored highly on questions pertaining to science-identity (3.8 ± 0.8 Full-Semester/Low Autonomy; 4.04 ± 0.77 Full-Semester/High Autonomy; 4.4 ± 0.69 Half-Semester/Low Autonomy), scientific community values (4.9 ± 0.9 Full-Semester/Low Autonomy; 5.11 ± 0.59 Full-Semester/High Autonomy; 5.13 ± 0.88 Half-Semester/Low Autonomy), project ownership of content (4.0 ± 0.6 Full-Semester/Low Autonomy; 3.92 ± 0.6 Full-Semester/High Autonomy; 0.88 ± 5.13 Half-Semester/Low Autonomy), and emotional project ownership (3.8 ± 0.6 Full-Semester/Low Autonomy; 3.78 ± 0.89 Full-Semester/High Autonomy; 4.12 ± 0.6 Half-Semester/Low Autonomy). While these preliminary results are promising, additional data collected from future implementations of this CURE will allow for a more thorough

analysis to evaluate the impact of the BBMP-CURE on student outcomes.

Despite the reported educational benefits of CUREs, challenges to their implementation persist (Spell et al., 2014; Cooper and Brownell, 2018). Logistical hurdles and lack of time to develop new laboratory research experiences are often cited as barriers that faculty face when considering a CURE (Spell et al., 2014; Shortlidge et al., 2016). The difficulties can be especially prominent for faculty members who work within primarily undergraduate institutions with limited access to research personnel and resources. The Bean Beetle Microbiome CURE overcomes these barriers as it is flexible, scalable, and utilizes supplies and techniques common for microbiology and biology classrooms. All protocols for the above modules are freely available at https://www.beanbeetles.org/workshops/curriculum-materials/ and https://www.beanbeetles.org/microbiome/resources-for-online-teaching/. Additionally, many of these protocols are also available in the *Proceedings of the Association for Biology Laboratory Education*, with detailed Instructor Notes and implementation guidance (Cole et al., 2018; Blumer and Beck, 2020), although the protocols presented here (and available on our website) offer revised and updated methods and notes based on participant feedback. The CURE has been designed such that educators have the freedom and flexibility to modify activities within the CURE to implement methods and techniques related to their own research interests. For example, during the cultivation of beetle-gut microbiome isolates, activities may be introduced that allow students to perform classical microbiological and microscopic analyses, such as differential staining (e.g., Gram-stain, acid-fast stain, endospore stain, etc.), hemocytometer-based microscopic cell counts of bacteria, and any number of physiological and metabolic assays. Additionally, the potential exists for students to conduct research that aligns with the independent research interests of the faculty. Thus, faculty have the opportunity to replicate studies from previous implementations, allowing future students to build on the findings from previous semesters, which can lead to more publishable work (LoSchiavo, 2018). Students and educators may take advantage of the current database of information on isolated microbes that is available on the Bean Beetle Microbiome Project website (beanbeetles.org), facilitating the generation of broader research questions across laboratory classrooms. Research on the bean beetle microbiome can be further facilitated by the open sharing of student-collected data. Currently, over 490 isolates are publicly available in the colony-based sequence and taxonomy databases at www.beanbeetles.org, and faculty are encouraged to submit their student-collected data to the database. While these data are publicly available, faculty who implement this CURE maintain ownership of the data collected by their students.

Another challenge of CUREs is that the authentic nature of the research means that, like with all research, students likely will carry out procedures that do not work in the first attempt. Research is inherently an iterative process where scientists must navigate scientific challenges, persevere through difficulties, and cope with failure (Henry et al., 2019). It has been argued that, for a research experience to be authentic, opportunities for iteration must be included (Corwin Auchincloss et al., 2014), as they allow for students to develop cognitive and emotional ownership of their work (Corwin et al., 2018). In the full-semester implementation of the Bean Beetle Microbiome CURE, iteration for procedures that commonly require multiple attempts (e.g., DNA extraction, PCR amplification) is built-in to the course schedule. A limitation of the half-semester implementation is that, due to the reduced time for completing the project, many iterative opportunities are not feasible.

The most significant barrier to incorporating microbiome research for undergraduate education is the cost associated with next-generation sequencing (NGS) (Hartman et al., 2016). This CURE outlines the use of the Illumina MiSeq System for paired-end sequencing of the beetle-gut microbiome. As NGS is now a relatively common technology, educators may have access to this or other sequencing platforms via their home universities at "in-house" costs, or may collaborate with colleagues who have access to NGS technology. Additionally, academic and commercial sequencing centers are available throughout the country that provide sequencing services and relatively low per-sample costs. The turn-around time for sequencing and data retrieval may vary, and educators interested in performing the NGS portion of this CURE should plan accordingly such that students have enough time to analyze data. Some sequencing centers provide data analysis for an additional cost, which may be of interest to educators whose desired learning outcomes are related to data interpretation as opposed to data processing. Additionally, the CURE is designed such that if NGS cost is prohibitive, a proxy for community analysis can be performed with phenotypic data of cultured isolates on solid media and colony-based PCR sequence data. Extensive sample datasets for both types of data are available in our open-access databases (Beanbeetles.org).

Other challenges may arise in the sessions dedicated to sequence data analysis using computational methods. Depending on the classroom set up, certain technical difficulties may arise (e.g., slow/unreliable internet connections, students who do not own personal laptops, students with different levels of familiarity with computers or spreadsheets). A teaching assistant or peer-mentor who can move around freely to aid students who may be struggling can be a valuable asset for these laboratory sessions. Additionally, access to university computational resources (e.g., computer labs) may alleviate some of the challenges of performing computational analysis in the laboratory classroom. For the DNA Subway portion of the bioinformatic analysis, students are encouraged to work in groups such that only one computer per group is logged on to the server to perform the metagenomic analysis, thereby reducing demands on the server that might prolong time to completion of the analysis. Alternatively, faculty may opt to have students perform the analyses as outside classwork so that students can have ample time to complete the analyses. Written and video tutorials are available to facilitate students' independent remote learning[3]. This option also allows

for class time to instead be dedicated to interpretation of graphs and data.

Finally, while the BBMP-CURE was originally designed for hands-on laboratory instruction, the global outbreak of COVID-19 has significantly altered current instructional programs. It is uncertain when and to what extent opportunities for in-person instruction will become available again for all undergraduates. The current situation facing universities underscores the need for quality classroom activities that can be performed in online and virtual formats. The work presented here adds value to the current state of instruction by providing easy-to-implement modules with access to our online-database of isolate and whole-community sequence datasets, which allows for virtual implementation of many aspects of this CURE.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Emory University Institutional Review Board (IRB00113934). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AZ performed literature review, created handouts and instructional materials, and wrote and edited majority of the manuscript. NG, LB, and CB created handouts and instructional materials and wrote and edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.577621/full#supplementary-material

FIGURE S1 | Survey data collected from faculty participants of the Bean Beetle Microbiome Project for the 2019–2020 academic year.

TABLE S1 | Course timeline for full-semester implementation.

TABLE S2 | Course timeline for half-semester implementation.

TABLE S3 | Example student and/or faculty-generated research questions implemented in the 2019–2020 academic year.

TABLE S4 | Preliminary results of group differences on the Laboratory Course Activities Survey (LCAS).

TABLE S5 | Mean and standard deviation for student responses from the Persistence in the Sciences (PITS) Survey.

REFERENCES

Agate, L., Beam, D., Bucci, C., Dukashin, Y., Jo'Beh, R., O'Brien, K., et al. (2016). The search for violacein-producing microbes to combat *Batrachochytrium dendrobatidis*: a collaborative research project between secondary school and college research students. *J. Microbiol. Biol. Educ.* 17, 70–73. doi: 10.1128/jmbe.v17i1.1002

Anderson, R. T., and Lovley, D. R. (1997). Ecology and biogeochemistry of in situ groundwater bioremediation. *Adv. Microb. Ecol.* 15, 289–350. doi: 10.1007/978-1-4757-9074-0_7

Bäckhed, F., Fraser, C. M., Ringel, Y., Sanders, M. E., Sartor, R. B., Sherman, P. M., et al. (2012). Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. *Cell Host Microbe* 12, 611–622. doi: 10.1016/j.chom.2012.10.012

Beck, C. W., and Blumer, L. S. (2007). "Bean beetles, *Callosobruchus maculatus*, a model system for inquiry-based undergraduate laboratories," in *Tested Studies for Laboratory Teaching*, ed. M. A. O'Donnell (Cupertino, CA: Association for Biology Laboratory Education (ABLE)), 274–283.

Blumer, L. S., and Beck, C. W. (2020). "Introducing community ecology and data skills with the bean beetle microbiome project," in *Advances in Biology Laboratory Education*, ed. K. McMahon (Cupertino, CA: association for Biology Laboratory Education (ABLE)).

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* 37, 852–857. doi: 10.1038/s41587-019-0209-9

Boomer, S. M., Lodge, D. P., and Dutton, B. E. (2002). Bacterial diversity studies using the 16S rRNA gene provide a powerful research-based curriculum for molecular biology laboratory. *Microbiol. Educ.* 3, 18–25. doi: 10.1128/154288102x14285807655107

Buck, L. B., Bretz, S. L., and Towns, M. H. (2008). Characterizing the level of inquiry in the undergraduate laboratory. *J. Coll. Sci. Teach.* 38, 52–58.

Chapelle, F. H. (2000). The significance of microbial processes in hydrogeology and geochemistry. *Hydrogeol. J.* 8, 41–46. doi: 10.1007/pl00010973

Colburn, A. (2000). An inquiry primer. *Sci. Scope* 23, 175–218.

Cole, M. F., Acevedo-Gonzalez, T., Gerardo, N. M., Harris, E. V., and Beck, C. W. (2018). "Effect of diet on bean beetle microbial communities," in *Tested Studies for Laboratory Teaching*, ed. K. McMahon (Cupertino, CA: Association for Biology Laboratory Education), 39.

Cooper, K. M., and Brownell, S. E. (2018). Developing course-based research experiences in discipline-based education research: lessons learned and

recommendations. *J. Microbiol. Biol. Educ.* 19:jmbe-19-88. doi: 10.1128/jmbe.v19i2.1567

Corwin, L. A., Graham, M. J., and Dolan, E. L. (2015a). Modeling course-cased undergraduate research experiences: an agenda for future research and evaluation. *CBE Life Sci. Educ.* 14, 1–13. doi: 10.1187/cbe.14-10-0167

Corwin, L. A., Runyon, C., Robinson, A., and Dolan, E. L. (2015b). The laboratory course assessment survey: a tool to measure three dimensions of research-course design. *CBE Life Sci Educ.* 14:ar37. doi: 10.1187/cbe.15-03-0073

Corwin, L. A., Runyon, C. R., Ghanem, E., Sandy, M., Clark, G., Palmer, G. C., et al. (2018). Effects of discovery, iteration, and collaboration in laboratory courses on undergraduates' research career intentions fully mediated by student ownership. *CBE Life Sci. Educ.* 17:ar20. doi: 10.1187/cbe.17-07-0141

Corwin Auchincloss, L., Laursen, S. L., Branchaw, J. L., Eagan, K., Graham, M., Hanauer, D. I., et al. (2014). Assessment of course-based undergraduate research experiences: a meeting report. *CBE Life Sci. Educ.* 13, 29–40. doi: 10.1187/cbe.14-01-0004

D'Avanzo, C. (1996). Three ways to teach ecology labs by inquiry: guided, open-ended, and teacher- collaborative. *Bull. Ecol. Soc. Am.* 77, 92–93.

Debelius, J. W., Vázquez-Baeza, Y., McDonald, D., Xu, Z., Wolfe, E., Knight, R., et al. (2016). Turning participatory microbiome research into usable data: lessons from the american gut project. *J. Microbiol. Biol. Educ.* 17, 46–50. doi: 10.1128/jmbe.v17i1.1034

Douglas, A. E. (2019). Simple animal models for microbiome research. *Nat. Rev. Microbiol.* 17, 764–775. doi: 10.1038/s41579-019-0242-1

Engel, P., and Moran, N. A. (2013). The gut microbiota of insects-diversity in structure and function. *FEMS Microbiol. Rev.* 37, 699–735. doi: 10.1111/1574-6976.12025

Finer, K. R., Fox, L., and Finer, J. J. (2016). Isolation and characterization of agrobacterium strains from soil: a laboratory capstone experience. *J. Microbiol. Biol. Educ.* 17, 444–450. doi: 10.1128/jmbe.v17i3.1124

Furgeson, L. V., Dhakal, P., Lebenzon, J. E., Heinrichs, D. E., Bucking, C., Sinclair, B. J., et al. (2018). Seasonal shifts in the insect gut microbiome are concurrent with changes in cold tolerance and immunity. *Funct. Ecol.* 32, 2357–2368. doi: 10.1111/1365-2435.13153

Garbarino, J., and Mason, C. E. (2016). The power of engaging citizen scientists for scientific progress. *J. Microbiol. Biol. Educ.* 17, 7–12. doi: 10.1128/jmbe.v17i1.1052

Gibbens, B. B., Scott, C. L., Hoff, C. D., and Schottel, J. L. (2015). Exploring metagenomics in the laboratory of an introductory biology course. *J. Microbiol. Biol. Educ.* 16, 34–40. doi: 10.1128/jmbe.v16i1.780

Goff, S. A. Vaughn, M., McKay S., Lyons, E., Stapleton, A. E., Gessler, D., et al. (2011). The iPlant collaborative: cyberinfrastructure for enabling data to discovery for the life science. *Front. Plant Sci.* 2:34. doi: 10.3389/fpls.2011.00034

Goodrich, J. K., Di Rienzi, S. C., Pool, A. C., Koren, O., Walters, W. A., Caporaso, J. G., et al. (2014). Conducting a microbiome study. *Cell* 158, 250–262. doi: 10.1016/j.cell.2014.06.037

Hammer, T. J., Janzen, D. H., Hallwachs, W., Jaffe, S. P., and Fierer, N. (2017). Caterpillars lack a resident gut microbiome. *PNAS* 114, 9641–9646. doi: 10.1073/pnas.1707186114

Hanauer, D. I., Frederick, J., Fotinakes, B., and Strobel, S. A. (2012). Linguistic analysis of project ownership for undergraduate research experiences. *CBE Life Sci. Educ.* 11, 378–385. doi: 10.1187/cbe.12-04-0043

Hanauer, D. I., Graham, M. J., Betancur, L., Bobrownicki, A., Cresawn, S. G., Garlena, R. A., et al. (2017). An inclusive research education community (iREC): impact of the SEA-PHAGES program on research outcomes and student learning. *PNAS* 114, 13531–13536. doi: 10.1073/pnas.1718188115

Hanauer, D. I., Graham, M. J., and Hatfull, G. F. (2016). A measure of college student persistence in the sciences (PITS). *CBE Life Sci. Educ.* 15:ar54. doi: 10.1187/cbe.15-09-0185

Hartman, M. R., Harrington, K. T., Etson, C. M., Fierman, M. B., Slonim, D. K., Walt, D. R., et al. (2016). Personal microbiomes and next-generation sequencing for laboratory based education. *FEMS Microbiol. Lett.* 363:fnw266. doi: 10.1093/femsle/fnw266

Henry, M. A., Shorter, S., Charkoudian, L., Heemstra, J. M., and Corwin, L. A. (2019). FAIL is not a four-letter word: a theoretical framework for exploring undergraduate students' approaches to academic challenge and responses to failure in STEM learning environments. *CBE Life Sci. Educ.* 18:ar11. doi: 10.1187/cbd.18-06-0108

Kandlikar, G. S., Gold, Z. J., Cowen, M. C., Meyer, R. S., Freise, A. C., Kraft, N. J. B., et al. (2018). ranacapa; An R package and Shiny web app to explore environmental DNA data with exploratory statistics and interactive visualizations. *F1000Research* 7:1734. doi: 10.12688/f1000research.16680.1

Kishbaugh, T. L. S., Cessna, S., Jeanne Horst, S., Leaman, L., Flanagan, T., Neufeld, D. G., et al. (2012). Measuring beyond content: a rubric bank for assessing skills in authentic research assignments in the sciences. *Chem. Educ. Res. Pract.* 13, 268–276. doi: 10.1039/C2RP00023G

Lentz, T. B., Ott, L. E., Robertson, S. D., Windsor, S. C., Kelley, J. B., Wollenberg, M. C., et al. (2017). Unique down to our microbes- assessment of an inquiry-based metagenomics activity. *J. Microbiol. Biol, Educ.* 18:18.2.33. doi: 10.1128/jmbe.v18i2.1284

Linnenbrink, M., Wang, J., Hardouin, E. A., Künzel, S., Metzler, D., Baines, J. F., et al. (2013). The role of biogeography in shaping diversity of the intestinal microbiota in house mice. *Mol. Ecol.* 22, 1904–1016. doi: 10.1111/mec.12206

LoSchiavo F. M. (2018). Incorporating a professional-grade all-class project into a research methods course. *Front. Psychol.* 9:2143. doi: 10.3389/fpsyg.2018.02143

Lopatto, D. (2003). The essential features of undergraduate research. *Counc. Undergrad. Res. Q.* 24, 139–142.

Lopatto, D. (2004). Survey of undergraduate research experiences (SURE): first findings. *Cell Biol. Educ.* 3, 270–277. doi: 10.1187/cbe.04-07-0045

Ma, Y., Chen, H., Lan, C., and Ren, J. (2018). Help, hope and hype: ethical considerations of human microbiome research and applications. *Protein Cell.* 9, 404–415. doi: 10.1007/s13238-018-0537-4

Martinez-Vaz, B. M., Denny, R., Young, N. D., and Sadowsky, M. J. (2015). An alternative approach to "identification of unknowns": designing a protocol to verify the identities of nitrogen fixing bacteria. *J. Microbiol. Biol. Educ.* 16, 247–253. doi: 10.1128/jmbe.v16i2.973

Merchant, N., Lyons, E., Goff, S., et al. (2016). The iPlant collaborative: cyberinfrastructure for enabling data to discovery for the life sciences. *PLoS Biol.* 14:e1002342. doi: 10.1371/journal.pbio.1002342

Mitchell, A. C., Dideriksen, K., Spangler, L. H., Cunningham, A. B., and Gerlach, R. (2010). Microbially enhanced carbon capture and storage by mineral-trapping and solubility-trapping. *Environ. Sci. Technol.* 44, 5270–5276. doi: 10.1021/es903270w

Muth, T. R., and McEntee, C. M. (2014). Undergraduate urban metagenomics research module. *J. Microbiol. Biol. Educ.* 15, 38–40. doi: 10.1128/jmbe.v15i1.645

National Academies of Sciences, Engineering, and Medicine (2017). *Undergraduate Research Experiences for STEM Students: Successes, Challenges, and Opportunities*. Washington, DC: The National Academies Press.

Nicholson, J. K., Holmes, E., and Wilson, I. D. (2005). Gut microorganisms, mammalian metabolism and personalized health care. *Nat. Rev. Microbiol.* 3, 431–438. doi: 10.1038/nrmicro1152

Rahman, S. J., Charles, T. C., and Kaur, P. (2016). Metagenomic approaches to identify novel organisms from the soil environment in a classroom setting. *J. Microbiol. Biol. Educ.* 17, 423–429. doi: 10.1128/jmbe.v17i3.1115

Seymour, E., Hunter, A. B., Laursen, S. L., and DeAntoni, T. (2004). Establishing the benefits of research experiences for undergraduates in the sciences: first findings from a three-year study. *Sci. Educ.* 88, 493–534. doi: 10.1002/sce.10131

Shortlidge, E. E., Bagnera, G., and Brownell, S. E. (2016). Faculty perspectives on developing and teaching course-based undergraduate research experiences. *BioScience* 66, 54–62. doi: 10.1093/biosci/biv167

Spell, R. M., Guinan, J. A., Miller, K. R., and Beck, C. W. (2014). Redefining authentic research experiences in introductory biology laboratories and barriers to their implementation. *CBE Life Sci. Educ.* 13, 102–110. doi: 10.1187/cbe.13-08-0169

Spor, A., Koren, O., and Ley, R. (2011). Unravelling the effects of the environment and host genotype on the gut microbiome. *Nat. Rev. Microbiol.* 9, 279–290. doi: 10.1038/nrmicro2540

Staub, N. L., Blumer, L. S., Beck, C. W., Delesalle, V. A., Griffin, G. D., Merritt, R. B., et al. (2016). Course-based science research promotes learning in diverse students at diverse institutions. *CUR Q.* 38, 36–46.

Surana, N. K. (2019). Moving microbiome science from the bench to the bedside: a physician-scientist perspective. *Msystems* 4:e00160-19. doi: 10.1128/mSystems.00160-19

Timmis, K., Cavicchioli, R., Garcia, J. L., Nogales, B., Chavarría, M., Stein, L., et al. (2019). The urgent need for microbiology literacy in society. *Environ. Microbiol.* 21, 1513–1528. doi: 10.1111/1452-2920.14611

Tuda, M., Roönn, J., Buranapanichpan, S., Wasano, N., and Arnqvist, G. (2006). Evolutionary diversification of the bean beetle genus *Callosobruchus* (Coleoptera: Bruchidae): traits associated with stored-product pest status. *Mol. Ecol.* 15, 3541–3551. doi: 10.1111/j.1365-294x.2006.03030.x

van der Heijden, M. G., Bardgett, R. D., and Van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* 11, 296–310. doi: 10.1111/j.1461-0248.2007.01139.x

Wang, J. T., Daly, J. N., Willner, D. L., Patil, J., Hall, R. A., Schembri, M. A., et al. (2015). Do you kiss your mother with that mouth? An authentic large-scale undergraduate re- search experience in mapping the human oral microbiome. *J. Microbiol. Biol. Educ.* 16, 50–60. doi: 10.1128/jmbe.v16i1.816

Wang, J. T. H. (2017). Course-based undergraduate research experiences in molecular biosciences-patterns, trends, and faculty support. *FEMS Microbiol. Lett.* 364:fnx157. doi: 10.1093/femsle/fnx157

Weaver, G. C., Russell, C. B., and Wink, D. J. (2008). Inquiry-based and research-based laboratory pedagogies in undergraduate science. *Nat. Chem. Biol.* 4, 577–580. doi: 10.1038/nchembio1008-577

Weber, C. F., and Werth, J. T. (2015). Culturing life from air: using a surface air system to introduce discovery-based research in aerobiology into the undergraduate biology curriculum. *J. Microbiol. Biol. Educ.* 16, 72–74. doi: 10.1128/jmbe.v16i1.813

Weber, K. S., Bridgewater, L. C., Jensen, J. L., Breakwell, D. P., Nielsen, B. L., Johnson, S. M., et al. (2018). Personal microbiome analysis improves student engagement and interest in immunology, molecular biology, and genomics undergraduate courses. *PLoS One* 13:e0193696. doi: 10.1371/journal.pone.0193696

Wong, C. N., Ng, P., and Douglas, A. E. (2011). Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. *Environ. Microbiol.* 13, 1889–1900. doi: 10.1111/j.1462-2920.2011.02511.x

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MicroMundo Upside Down: Targeted Searching for Antibiotics-Producing Bacteria From Soil With Reverse Antibiosis Approaches

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Tiny Earth (TE) is a popular international citizen science program aimed at improving public awareness on the growing antimicrobial resistance problem of which MicroMundo Albacete is a Spanish node. With a protocol that is focused on the isolation of antibiotics-producing actinomycetes from soil, 70% of the high school students in MicroMundo Albacete 2020 isolated colonies with antagonistic activity against Gram-positive tester bacteria. However, no activity was found against Gram-negative bacteria. Here, we further adapted the protocol toward a more targeted screening that also enables isolation of antagonistic bacteria against Gram negatives using two different reverse-antibiosis approaches involving a spraying technique or flipping soil sample disks upside down. Exploiting the soil samples from MicroMundo Albacete 2020, the new approaches yielded isolation of actinomycete bacteria with antagonistic activity against Gram-negative as well as Gram-positive tester bacteria. We propose that (educational) science programs which aim to search for antibiotic-producing bacteria may implement these approaches in their protocol to promote a successful and stimulating outcome of the experiment for the participating students.

Keywords: antibiotics, antimicrobial resistance, actinomycetes, ESKAPE pathogens, Tiny Earth, MicroMundo, service-learning

INTRODUCTION

Continued emergence of multidrug-resistant bacteria is considered a growing and global human health threat. The number of new antibiotics that have been launched in recent years, especially those with activity against Gram-negative bacteria, is very limited and seems insufficient to combat the so-called "antibiotic crisis" (Wright, 2015; Butler et al., 2017; Van Puyvelde et al., 2018). As a consequence, toward the second half of the 21st century, this may bring our vulnerability for acquiring lethal microbial infections back to the situation how it was before the discovery of antibiotics (Leung et al., 2011; Laxminarayan et al., 2013; O'Neill, 2016). On the positive side, governmental regulations on the use of antibiotics in many developed countries have recently become stricter and have forbidden their application in poultry feed.

Nevertheless, crucial to prevent a devastating future perspective is to achieve a better public awareness on the proper use antimicrobials. Tiny Earth (TE) is a very successful and popular crowdsourcing (also named studentsourcing or citizen science) program that is implemented worldwide to address the antimicrobial drug resistance (AMR) issue (Hernandez et al., 2018). The basic idea of the program is to enhance awareness on the AMR problem among students by letting them participate in a real and exciting experiment that is aimed at antibiotic discovery. In Spain, the program, named MicroMundo, is run in a service-learning set-up in which undergraduate students, after receiving training by academic researchers, perform as teaching assistants during teaching sessions with high school classes (Valderrama et al., 2018; De Groot et al., 2019). When running the program for the first time at the University of Castilla-La Mancha (UCLM) in Albacete in 2019, we decided to focus the experimental protocol on isolation of Gram-positive actinomycetes, the main producers of most clinically used antibiotics (Procópio et al., 2012; Barka et al., 2016; Butler et al., 2017). Doing so, a higher percentage of the isolated bacteria with antagonistic activity against tester bacteria was obtained, making the project more alluring for the participating students (De Groot et al., 2019). However, with this strategy, it appears more likely to pick up antagonistic activity against Gram-positive than against Gram-negative bacteria as all of the positive isolates produced antagonistic activity against Gram positives but no activity against Gram-negative tester bacteria was observed (De Groot et al., 2019).

To implement a more targeted screening that will make the protocol more amenable to also pick up activity against Gram-negative bacteria, we, here, designed a further modification to the protocol, functionality of which is demonstrated with soil samples from the MicroMundo 2020 project.

MATERIALS AND METHODS

MicroMundo Albacete 2020

The set-up including methodological details of the MicroMundo Albacete service-learning project is described in De Groot et al. (2019). In 2020, the program was run at two high schools [IES Tomás Navarro Tomás (TNT) and IES Andrés de Vandelvira (AV)]. Twenty student pairs each analyzed 10 colonies from self-taken soil samples (taken in the provinces of Albacete and Cuenca) using two different growth media [Reasoner's 2A agar (R2A, Oxoid) in TNT and Actinomycete Isolation Agar (AIA, Sigma-Aldrich) in AV] that favor growth of actinomycetes. Both media were supplemented with the antimicrobials dieldrin (4 mg/L), nalidixic acid (20 mg/L), and cycloheximide (80 mg/L) to inhibit growth of mites, Gram-negative bacteria, and fungi, respectively. The final antibiosis experiment of the purified bacterial isolates was performed with Gram-positive tester bacteria *Bacillus subtilis* (strain ATCC 6051) and *Staphylococcus epidermidis* (ATCC 14990), and Gram-negative *Escherichia coli* (ATCC 11775). The experiment was repeated by experienced UCLM researchers to validate the antagonistic activity.

Reverse Antibiosis Approaches

Aiming to achieve a more targeted screening of soil samples, two alternative reverse antibiosis approaches were developed: (i) plates grown with serial dilutions of soil samples, containing up to a few hundred colonies, were sprayed with 0.4–0.5 ml of ESKAPE-relative tester bacteria culture, freshly grown overnight in rich medium [LB Broth (Fisher Scientific) or Tryptic Soy Broth (TSB, Oxoid) at 30°C], using a spray bottle. (ii) Fully grown (10 days) soil sample agar plates were loosened from their Petri dishes and placed upside down in the lid of the dish. The bottom side now facing up was inoculated with a tester bacterium by painting the entire surface of the plate using a sterile cotton swab (Deltalab). In both methods, appearance of halos is monitored after 1–3 days of incubation at 30°C. Candidate bacterial colonies were purified by re-streaking on R2A or AIA plates with and without nalidixic acid, and their potential antagonistic activity was verified using the same antibiosis technique as in the MicroMundo project (De Groot et al., 2019) but using the original media (R2A and AIA) instead of TSB agar (TSA).

Molecular Identification

The methodology for genomic DNA isolation of purified bacteria is described in De Groot et al. (2019). Amplification of the complete 16S rDNA gene was achieved using oligonucleotides fD1: 5'-AGAGTTTGATCCTGGCTCAG-3' and Rp2: 5'-ACGGC TACCTTGTTACGACTT-3' (Weisburg et al., 1991), purchased from STAB-Vida. PCR was performed using a proofreading KAPA HiFi PCR kit (KAPA Biosystems) following the manufacturer's instructions. PCR fragments were checked on agarose gel, cleaned up (ExtractMe DNA clean-up kit, BLIRT), and sequenced (STAB-Vida). Primers 16S_339_F and 16S_1087_R (De Groot et al., 2019), were also used for sequencing. Taxonomic and phylogenetic DNA sequence analysis was performed using NCBI-Blast and MEGA X (Kumar et al., 2018) software.

RESULTS AND DISCUSSION

In the MicroMundo Albacete 2020 project, 200 selected bacterial isolates were tested for antagonistic activity against ESKAPE-like tester bacteria by high school students. The acronym ESKAPE refers to six leading nosocomial pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) that exhibit multidrug resistance and virulence (Mulani et al., 2019). For biosafety reasons, in the studentsourcing program, these pathogenic bacteria are replaced by harmless "relatives." Guided by results of the previous year (De Groot et al., 2019), we tested antagonistic activity against the Gram-positive ESKAPE-relatives *B. subtilis* and *S. epidermidis*, and against Gram-negative *E. coli*. Twenty-five of the isolates (12.5%) showed antagonistic activity against the Gram-positive *B. subtilis* and *S. epidermidis* (**Supplementary Table S1**), whereas no growth-inhibiting activity was detected against Gram-negative *E. coli*. Fourteen of the 20 (70%) student pairs detected at least one positive isolate in their soil samples. To demonstrate that positive isolates are (mostly) actinomycetes, 16S rDNA of isolates from 13 different soil samples

was sequenced, in each case revealing highest identity levels with different *Streptomyces* species (**Supplementary Table S1**). This is not an unexpected result. Like many other *Streptomyces* spp. (Bagley et al., 2005), several of them are already known as producers of different types of antibiotics (**Supplementary Table S1**), and most antibiotics obtained from actinomycetes originate from *Streptomyces* spp. (Berdy, 2005; Barka et al., 2016). We consider the results of MicroMundo Albacete 2020 very positive and consistent with those of the previous year (De Groot et al., 2019). Once again it demonstrated that with a protocol directed toward isolation of actinomycetes, the majority of the high school students were able to isolate antibiotic-producing bacteria from soil.

However, as in the previous year, antagonistic activity against Gram-negative bacteria was not discovered during MicroMundo Albacete 2020, and this seems an undesired limitation of the chosen approach. Further, the MicroMundo project highly relies on serendipity as a limited number of actinomycete-like colonies (often 10) from a given soil sample are more or less randomly chosen for further purification and antibiosis tests. Aiming to improve the protocol on both aspects, we decided to exploit the soil samples of the MicroMundo project to develop two different reverse antibiosis approaches.

Approach 1: Spraying Technique

Some of the plates from which the students had selected their candidate colonies were carefully sprayed with an aliquot of fresh cultures of ESKAPE-relative tester bacteria. Prior growth tests indicated that *Pseudomonas putida* is the only Gram-negative ESKAPE-relative that can grow on media containing 20 μg/ml nalidixic acid (as present in the soil sample plates). After spraying *P. putida* and allowing time for growth, on about 25% of the soil sample plates containing up to a few hundred colonies, one or two colonies were apparently surrounded by small, but sometimes larger, halos (**Figure 1A**).

The spraying technique subsequently requires thorough purification of positive bacteria by re-streaking to eliminate the contaminating tester bacteria. Purification of positive candidates is in progress. Antibiosis testing of three already purified bacteria on the original R2A or AIA medium (without antibiotics) confirmed antagonistic activity of two of these isolates against *P. putida* (**Figure 1B**). Interestingly, both bacteria also showed slight antagonistic activity against other, Gram-positive and Gram-negative, ESKAPE-relative tester bacteria (**Table 1**). DNA sequencing revealed that both positive isolates are *Streptomyces* spp. (**Table 1**). Thus, the spraying technique allows for targeted screening of actinomycetes with antagonistic activity against Gram-negative bacteria in soil samples.

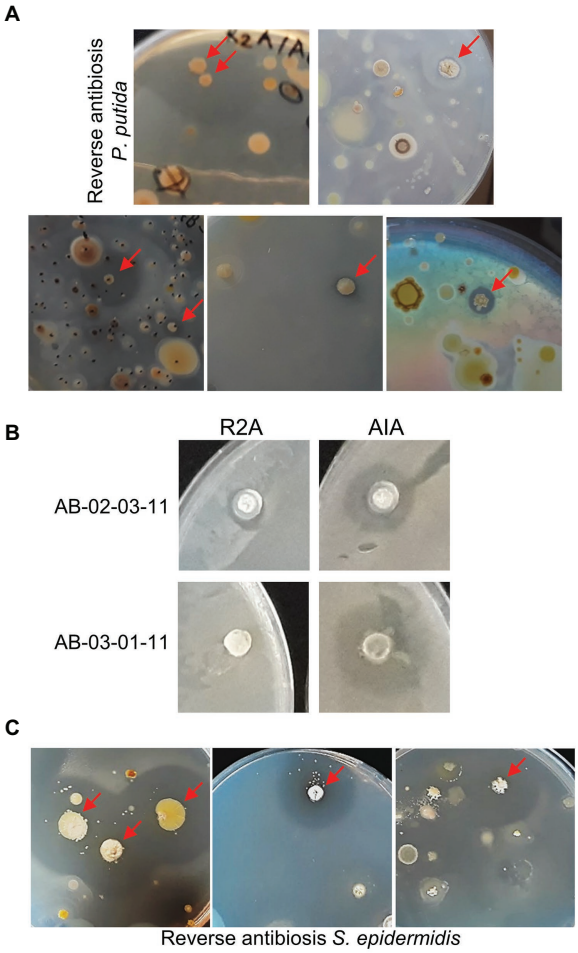

FIGURE 1 | Reverse antibiosis spraying technique. **(A)** Examples of soil sample plates sprayed with the Gram-negative bacterium *Pseudomonas putida*. Colonies surrounded by halos are indicated by arrows. **(B)** Confirmative *P. putida* antibiosis testing of two purified positive colonies on R2A and Actinomycete Isolation Agar (AIA). **(C)** Examples of soil sample plates sprayed with the Gram-positive bacterium *Staphylococcus epidermidis*.

TABLE 1 | Isolated antibiotic-producing bacteria using reverse antibiosis approach with *P. putida*.

Soil sample code	Growth medium	Size of halo (mm)[a]				NCBI-Blast result
		Bs[b]	Se	Pp	Ec	
		R2A/AIA	R2A/AIA	R2A/AIA	R2A/AIA	
AB-02-03-11	R2A + NA[b]	11/8	nh[a]/nh	8/13	nh/9	99.0% id. with *S. vastus* NBRC 13094
AB-02-03-12	R2A + NA	No antagonistic activity in antibiosis tests				
AB-03-01-11	AIA + NA	9/7	nh/10	9/17	nh/nh	99.9% id. with *S. gardneri* B547

[a]*Size of agar disk is 6 mm. nh, no halo.*
[b]*Bs, B. subtilis; Se, S. epidermidis; Pp, P. putida; Ec, E. coli; NA, nalidixic acid.*

In soil samples plated on R2A or AIA medium, colonies with antagonistic activity against Gram-positive bacteria are often abundant and even by random selection, it proves relatively easy for the high school students to find some positives. However, if one applies other growth conditions, this may be more challenging. Therefore, targeted screening using the spraying technique was also attempted using *S. epidermidis*, the only Gram-positive ESKAPE-relative that can grow, albeit very slowly, on R2A and AIA in the presence of 20 μg/ml nalidixic acid, as tester bacterium. Some soil samples that were shown to contain positive isolates in the MicroMundo project were re-plated on R2A or AIA media and sprayed with *S. epidermidis*. As expected, colonies surrounded by clear halos were observed already on plates containing only few colonies (**Figure 1C**). We conclude that the reverse antibiosis spraying method allows for a targeted screening of bacteria with antagonistic activity against Gram-positive as well as Gram-negative bacteria.

Approach 2: Flipping Technique

Although proving efficient, the spraying technique has two drawbacks: (i) for safety reasons, the procedure should be performed in a flow cabinet, which normally is not available in high school laboratories and (ii) positive bacteria are contaminated with tester bacteria by spraying and need purification. Therefore, as an alternative approach, reverse antibiosis was attempted by placing soil sample agar disks upside down in the lid of the Petri dish, and seeding tester bacteria on the bottom surface of the disks (now facing upwards) with a cotton swab. Validity of this approach was first tested with some purified actinobacteria identified in the MicroMundo project, that were seeded with *S. epidermidis* or *B. subtilis*, and with the two purified bacteria showing activity against *P. putida*, that were seeded with *P. putida* (**Figure 2A**). The flipping method was then applied to three MicroMundo soil samples (AB-02-06, AB-02-10, and AB-03-07) that contained ample antagonistic activity as identified by the high school students. At dilution 10^{-6}, with only few colonies grown on the plates, about 50% of them already showed colonies surrounded by halos when probed with *S. epidermidis* (examples in **Figure 2B**), demonstrating the potential of the method. However, soil samples grown on R2A and AIA normally also contain quite a few soft and slimy colonies in addition to actinomycetes. After flipping the plate, such colonies get squeezed between agar and plate during the inoculation with tester bacteria, causing their spread and contamination of other colonies. This complicates screening and isolation of colonies on densely populated plates. Currently, tests in our laboratory are in progress to incorporate a simple heat treatment before plating to eliminate soft colonies from non-spore forming bacteria and allow enrichment of actinobacteria (Tiwari and Gupta, 2013). A test with applying heat (60°C for 20–60 min) to soil samples was found to reduce the number of colonies on the plates by about 90%. With a soil sample (AB-03-08) that had not yielded positive bacteria in the MicroMundo project, screening the heat-treated sample with the flipping method identified colonies with activity against *S. epidermidis*, as well as *P. putida* (**Figure 2C**). Thus, the pre-treatment makes the flipping technique suitable for screening, including identification of more rare activity against Gram-negative bacteria on densely populated plates. We, therefore, believe that this will become the preferred method for

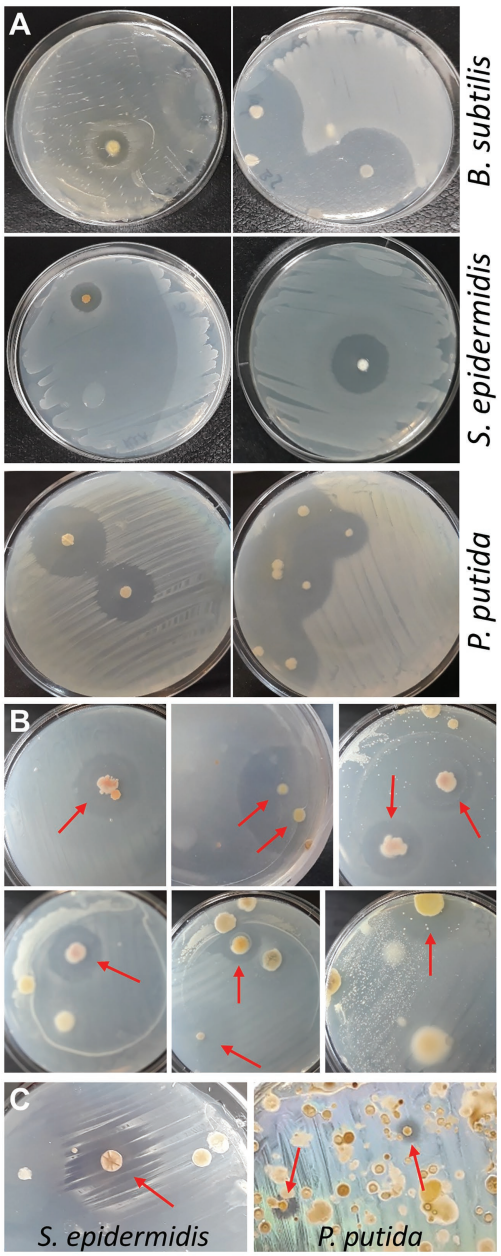

FIGURE 2 | Reverse antibiosis flipping technique. **(A)** Testing of reverse-antibiosis flipping method using plates with purified positive actinobacteria. After flipping the agar upside down, plates were seeded with tester bacteria. Plates with purified positive actinobacteria from the MicroMundo 2020 project were seeded with *Bacillus subtilis* or *S. epidermidis*. Plates with purified actinobacteria that were identified to have activity against *P. putida* by the spraying method were seeded with *P. putida* (left, AB-02-03-11; right, AB-03-01-11). **(B)** Cultivated soil samples at dilutions 10^{-6} were flipped and seeded with the Gram-positive bacterium *S. epidermidis*. Colonies surrounded by halos are indicated by arrows. **(C)** Heat-treated soil sample AB-03-08, plated and grown on R2A and AIA, was flipped and seeded with *S. epidermidis* (left; R2A, dilution 10^{-3}) and *P. putida* (right; AIA, dilution 10^{-1}).

implementation in the educational MicroMundo program, as no special equipment or skills are required.

In conclusion, we have developed two reverse antibiosis approaches for targeted screening of actinobacteria that are easily applicable to identify antagonistic activity against Gram-negative as well as Gram-positive bacteria from soil.

DATA AVAILABILITY STATEMENT

DNA sequences presented in this study can be found in the online repository GenBank under accession numbers MT992770–MT992784. Results of MicroMundo Albacete 2020 are detailed in **Supplementary Material**.

ETHICS STATEMENT

Ethical review and approval was not required for the human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

PG, PC-C, and DM designed the study. PG and MA wrote the paper. PG, MA, and DM performed the experiments. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.577550/full#supplementary-material

REFERENCES

Bagley, M. C., Dale, J. W., Merritt, E. A., and Xiong, X. (2005). Thiopeptide antibiotics. *Chem. Rev.* 105, 685–714. doi: 10.1021/cr0300441

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Meier-Kolthoff, J. P., et al. (2016). Taxonomy, physiology, and natural products of actinobacteria. *Microbiol. Mol. Biol. Rev.* 80, 1–43. doi: 10.1128/MMBR.00019-15

Berdy, J. (2005). Bioactive microbial metabolites. *J. Antibiot.* 58, 1–26. doi: 10.1038/ja.2005.1

Butler, M. S., Blaskovich, M. A., and Cooper, M. A. (2017). Antibiotics in the clinical pipeline at the end of 2015. *J. Antibiot.* 70, 3–24. doi: 10.1038/ja.2016.72

De Groot, P. W. J., Fernández-Pereira, J., Sabariegos, R., Clemente-Casares, P., Parra-Martínez, J., Cid, V. J., et al. (2019). Optimizing Small World Initiative service learning by focusing on antibiotics-producing actinomycetes from soil. *FEMS Microbiol. Lett.* 366:fnaa019. doi: 10.1093/femsle/fnaa1019

Hernandez, S., Tsang, T., Bascom-Slack, C., Broderick, N., and Handelsman, J. (2018). *Tiny Earth—A research guide to studentsourcing antibiotic discovery. 18th Edn*. Ann Arbor, MI: XanEdu Publishing Inc.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547–1549. doi: 10.1093/molbev/msy096

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K., Wertheim, H. F., Sumpradit, N., et al. (2013). Antibiotic resistance-the need for global solutions. *Lancet Infect. Dis.* 13, 1057–1098. doi: 10.1016/S1473-3099(13)70318-9

Leung, E., Weil, D. E., Raviglione, M., and Nakatani, H. (2011). The WHO policy package to combat antimicrobial resistance. *Bull. World Health Organ.* 89, 390–392. doi: 10.2471/BLT.11.088435

Mulani, M. S., Kamble, E. E., Kumkar, S. N., Tawre, M. S., and Pardesi, K. R. (2019). Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. *Front. Microbiol.* 10:539. doi: 10.3389/fmicb.2019.00539

O'Neill, J. (2016). *Antimicrobial resistance: Tackling a crisis for the health and wealth of nations*. London, England: Review on Antimicrobial Resistance.

Procópio, R. E., Silva, I. R., Martins, M. K., Azevedo, J. L., and Araújo, J. M. (2012). Antibiotics produced by *Streptomyces*. *Braz. J. Infect. Dis.* 16, 466–471. doi: 10.1016/j.bjid.2012.08.014

Tiwari, K., and Gupta, R. K. (2013). Diversity and isolation of rare actinomycetes: an overview. *Crit. Rev. Microbiol.* 39, 256–294. doi: 10.3109/1040841X.2012.709819

Valderrama, M. J., González-Zorn, B., de Pablo, P. C., Díez-Orejas, R., Fernández-Acero, T., Gil-Serna, J., et al. (2018). Educating in antimicrobial resistance awareness: adaptation of the Small World Initiative program to service-learning. *FEMS Microbiol. Lett.* 365:fny161. doi: 10.1093/femsle/fny1161

Van Puyvelde, S., Deborggraeve, S., and Jacobs, J. (2018). Why the antibiotic resistance crisis requires a One Health approach. *Lancet Infect. Dis.* 18, 132–134. doi: 10.1016/S1473-3099(17)30704-1

Weisburg, W. G., Barns, S. M., Pelletier, D. A., and Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* 173, 697–703. doi: 10.1128/jb.173.2.697-703.1991

Wright, G. D. (2015). Solving the antibiotic crisis. *ACS Infect. Dis.* 1, 80–84. doi: 10.1021/id500052s

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METHODS
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There're CRISPRs in My Yogurt: A Discovery-Based CURE at the Intersection of Industrial Food Production and the Human Microbiome

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Support for undergraduate laboratory education based on a CURE (Course-based Undergraduate Research Experience) model is more widespread than ever. By giving students the opportunity to conduct genuine research in laboratory courses they are required to take, CUREs can expose more students to scientific practice and have the potential to make science more inclusive, especially when research topics have direct impact on students' lives. Here, I present a new microbiology CURE module where students explore the real-world intersection between industrial food production and the human microbiome. In this module, students sequence CRISPR arrays in the genomes of lactic acid bacteria they isolate from yogurt. Natural CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) act as the bacterial immune system. When a bacterial cell survives viral infection, it can incorporate a bit of that virus's DNA into its own genome, and produce small RNA guides that surveil the cell, ready to deploy virus-destroying enzymes if matching DNA from a fresh viral infection is detected. This viral immunity is of particular interest in the fermentation industry, since viral infection can destroy stocks of starter cultures and batches of product. Commercial producers of lactic acid bacteria for yogurt production often endeavor to produce strains with large CRISPR arrays and robust immunities. With this context, students are given the task of cataloging the viral immunities found in both commercial and traditionally produced yogurt, and exploring their potential impact on human health. Wet-lab practices (strain isolation, PCR, and Sanger sequencing) are combined with bioinformatic and literature sleuthing to identify the viruses to which bacteria are immune and explore whether consumption of these strains could impact human health via interactions with the human microbiome. Here, a detailed implementation of the module is presented with guides for educators and students.

Keywords: lactic acid bacteria, yogurt, gut microbiome, CRISPR, bacteriophage resistance, CURE, inquiry-based lab

INTRODUCTION

Educational Context

In the past decade or two there has a been an increasing call to make the science students learn in laboratory courses more reflective of the way science is actually conducted (American Association for the Advancement of Science [AAAS], 2011). One way to do this is through research projects that are open-ended and more authentic than standardized cookbook style activities. In these projects, not only do the students not know what the results might be, but neither does the instructor, nor the scientific community at large. Such projects, where the results have potential novel relevance to science, have been dubbed CURES — Course-based Undergraduate Research Experiences (Auchincloss et al., 2014). CURES can make science more inclusive by providing research opportunities for students who, due to economic hardship or other systemic barriers, are unable to obtain a traditional, apprenticed, extracurricular research position in a laboratory (Bangera and Brownell, 2017).

Because of these potential benefits, CUREs have become increasingly widespread. One of the most well-known CUREs, the nationwide SEA-PHAGES project, first got its start in the early 2000s (Hanauer et al., 2006). Since that time, countless CURES have been developed, both by individual instructors and as multi-campus initiatives. Along with the increased development and adoption of CUREs, a large body of work has been produced that studies their impact on students. These studies aim to identify which components of CUREs make them beneficial to students, and also provide a framework for how CUREs may benefit students by increasing persistence in science.

What has this work shown? In 2014, a working group from the NSF-funded Course-Based Undergraduate Research Experience Network (CUREnet) published an operational definition of a CURE, based on five components, or dimensions, of research (Auchincloss et al., 2014). According to the CUREnet report, authentic CURES that represent genuine research should embody all five dimensions (**Table 1**). How do these dimensions benefit students? Participation in course-based research has been shown to improve student understanding of the nature of science (Russell and Weaver, 2011) and promote scientific thinking as well as the ability to interpret data (Brownell et al., 2017). In addition to these cognitive gains, studies have also looked at the affective and psychosocial impacts of student participation in CUREs. Work has shown that project ownership, self-efficacy, science identity, and adoption of scientific community values can be influenced by CUREs (Hanauer et al., 2016; Cooper et al., 2019). Students who measure higher in these areas are more likely to persist in science, and this is especially true for students from underrepresented populations (Hanauer et al., 2016; Corwin et al., 2018). CURE researchers are still pursuing a full understanding of how individual CURE components and activities contribute to these outcomes, how much of each component in a course is sufficient to see gains, and how different student populations may see different impacts (Auchincloss et al., 2014).

TABLE 1 | The five dimensions of research in CUREs and how they are represented in this module.

	Dimension of research	Representation in "There're CRISPRs in my yogurt" module
1	Multiple scientific practices (students do more than just collect data)	Students collect data according to project's scientific goals, but they also analyze data, and in lab reports, interpret data, communicate their findings, and propose future experiments.
2	Discovery (results are unknown to students and instructors)	CRISPR arrays of commercial and heirloom yogurt bacterial strains have not been compared, and it is unknown if and how consuming strains with altered viral immunities may affect the gut microbiome.
3	Relevance (there is potential for broad relevance beyond the scope of the course)	Food microbiology and the human gut microbiome are fields with broad general interest; there are many unanswered questions in our understanding of interactions between diet and the gut microbiome.
4	Collaboration	Students collaborate on multiple levels: they coordinate with their partner and group to carry out experiments, and use shared data from the entire class to draw conclusions.
5	Iteration	Students carry out repeated screens of multiple strains, and the module builds in time to repeat experiments if there are failures. There is also iteration from course to course, as lessons are learned and new strains are isolated and analyzed.

Dimensions adapted from CUREnet working group report (Auchincloss et al., 2014).

This paper presents a CURE module, "There're CRISPRs in my Yogurt" that could be incorporated into an undergraduate microbiology laboratory course. The module would be most appropriate for junior or senior students.

Scientific Context

The goals of this and most CUREs are two-fold. The first goal is to give students exposure to genuine research and thereby give them a better understanding of how new knowledge is constructed. The second goal is to contribute to scientific understanding. Here, the CURE explores CRISPR-based viral immunity in lactic acid bacteria used to produce yogurt for consumption. The ultimate goal is to create a catalog of viral immunity in food strains, which can be used as a reference to better understand potential interactions between the gut microbiome and the foods we eat.

While many students have heard of CRISPR in the context of its use in genetic engineering, they are less aware of the function of natural CRISPRs. Researchers first observed the pattern of CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) in *E. coli* (Ishino et al., 1987). In a given bacteria, they consist of arrays of conserved repeat sequences of ∼21–40 bp, separated by non-conserved spacer sequences of ∼20–58 bp (Ishino et al., 2018). The key moment in discerning their function came when researchers noticed that the spacer sequences matched foreign DNA sources — bacteriophage, prophages, and phagemids — and that bacteria which harbored spacers were resistant to infection by the

corresponding virus (Mojica et al., 2005; Pourcel et al., 2005). This immunologically protective function of CRISPRs was experimentally confirmed in *Streptococcus thermophilus*, by researchers affiliated with Danisco, a company producing bacterial starters for the food industry (Barrangou et al., 2007; Lander, 2016).

The molecular details of how cas proteins incorporate bits of viral DNA into the host CRISPR array and then use guide RNAs derived from them to target foreign DNA in subsequent infections were figured out over the next decade (Lander, 2016; Ishino et al., 2018). Phage-resistant bacteria are of interest to companies that produce lactic acid bacteria strains for the dairy industry, since phage infection can ruin industrial cultures (Lander, 2016). Since the discovery of their function, commercial companies have systematically created phage-resistant bacterial strains by a process akin to vaccination: bacteria are exposed to phage, and surviving bacteria are screened for the presence of CRISPRs (Grens, 2014; Barrangou and Horvath, 2017).

Because of their different natural histories, and because some commercial strains may have been intentionally vaccinated, the CRISPR loci of industrially produced commercial yogurt bacteria may differ, in both size and spacer identity, from the CRISPR loci of traditionally produced heirloom yogurt. Modern commercial yogurt is produced by fermentation of milk with a few well-defined isogenic strains. Here, heirloom yogurt refers to yogurt made from a mother culture containing an undefined mixture of wild strains (Fisberg and Machado, 2015). Yogurt is generally lauded for its broad health benefits (Fisberg and Machado, 2015), and its potential to positively influence the human gut microbiome (Veiga et al., 2015), but interactions of yogurt bacteria with the commensal microbiome are not fully characterized.

The human gut microbiome includes a bacteriophage component which varies from person to person; persistent phage can be linked to abundant host bacteria in the gut (Shkoporov et al., 2019). Phage may play key roles in regulating bacterial abundance in the microbiome. If bacteria with CRISPRs that render them resistant to these phage are consumed, their resistance could potentially spread to the commensal bacteria via horizontal gene transfer (Godde and Bickerton, 2006; Horvath et al., 2009), leading to dysbiosis. Another role for phage in the human microbiome is as an antibacterial layer that protects mucosal surfaces (Barr et al., 2013; Barr, 2017). If phage play this role in the gut of healthy individuals, consumption of bacteria resistant to these phage could also lead to dysbiosis and potential bacterial infiltration of epithelial cells (Ogilvie and Jones, 2015). Data gathered in this CURE could help illuminate the potential for these types of interactions by identifying the bacteriophages that commonly consumed lactic acid bacterial strains are immune to.

Potential Student Outcomes

In this methods article we provide guidelines to instructors who would like to incorporate this CURE as a module in a microbiology lab course. A brief, 2–3 weeks module like this can be an accessible way to begin to bring open-ended research into an existing class, and the specific topic should be of broad interest to students. Students who can see connections between the science they are learning and the real-world perform better and are more likely to persist in science (Cromley et al., 2016), particularly if they can see how the science may be personally relevant to their lives, which may be especially important for underrepresented minorities (Hurtado et al., 2010). By connecting hands-on research with a question relevant to the everyday lives of students and their communities (what people eat and how it can affect them), this module aims to stoke students' interest and engagement with science, and contribute to a reduction in achievement gaps.

How do we hope to achieve these goals? This module brings authentic research to students as part of their normal curriculum. Though there are various frameworks for evaluating the authenticity of research experiences (reviewed in Rowland et al., 2017), we have taken advantage of the simplicity of the CUREnet five dimensions framework (Auchincloss et al., 2014) and have mapped how this module embodies each dimension in **Table 1**. We have only begun to assess the impact of this module on students. However, in the future we hope to assess cognitive, affective, and psychosocial outcomes, and have suggested instruments to do so in the discussion.

METHODS FOR IMPLEMENTING MODULE AS PART OF A COURSE

Implementation of this module requires six active lab sessions, taking a maximum of 3 h each (see **Figure 1**). Some of the sessions do not require all 3 h, so they could be combined with lectures to provide key background information or discussions where students share results with one another. The following sections provide a general lab guide for instructors, covering key considerations and pitfalls to avoid for each step of the module. Detailed, step-by-step instructions for students are provided in the supplementary manual, which also includes background information and explanation of the protocols. To expedite time in the lab, we usually pre-aliquot the reagents required by each group of students; we usually provide a little extra volume than what is specified in the **Supplementary Material**.

Producing Heirloom Yogurt

Heirloom yogurt contains an undefined mixture of microbes originally derived from the environment. If instructors have access to a heirloom yogurt culture at home, or from a friend or relative, it could certainly be used for this module and propagated indefinitely. However, not everyone has access to a home-grown heirloom culture. Fortunately, the popularity of home-fermentation has resulted in some commercially available heirloom cultures.

In our implementation of the class, we use two heirloom yogurt starters available from the company Cultures for Health

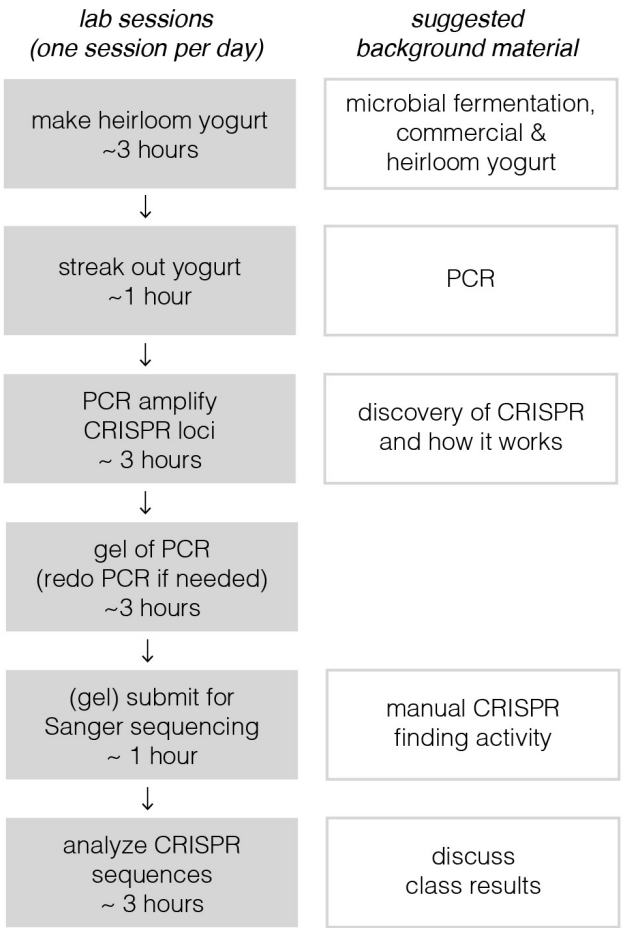

FIGURE 1 | Breakdown of lab sessions and suggested lessons for downtime or lectures in between labs. On the left, each lab session, constrained by a bubble, is designed to be carried out on a single lab day. Sessions are broken up to allow for overnight incubation steps or when tasks would take too long for students to complete in a 3-h window. Depending on course frequency, these six sessions, occurring on six separate lab days, would likely take 2–3 weeks at most institutions. This could in principle be shortened to as little as four consecutive days. With a full-day lab session, the third, fourth, and possibly even the fifth sessions could be combined. Timing for the last session depends on how quickly sequencing results could be returned; there are many same-day and next-day services available.

(2020). It is important to select starters that are actually labeled (in the product name or in the description) as "heirloom;" the company also sells conventional, defined-species starters (which they somewhat confusingly sometimes refer to as "traditional" in that, for most of the past century, defined-strain yogurts were the only ones commercially available in the United States). Starters are supplied as a dried powder; we pre-aliquot 0.8 g of the powder (1/10th of the packet) into individual microfuge tubes so the students can then easily tap out the entire contents of the microfuge tube into 100 mL of scalded milk. (To scald the milk, it is poured into individual Pyrex bottles with loose lids, brought to 82–85°C in a water bath and held for 10 min, then stored at 4°C. Prior to class, it is pre-warmed to 43°C).

Isolating Potential *S. thermophilus* Colonies

Both heirloom and commercial yogurts may be made with a wide variety of lactic acid bacteria, however there are two species that are most prevalent. These are *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. They have a symbiotic relationship in yogurt cultures (reviewed in Aryana and Olson, 2017), and all commercial yogurt sold in the United States is required to be made with at least these two species. To facilitate comparisons between different yogurts, the module focuses exclusively on these two strains. While we have amplified CRISPR regions from *L. bulgaricus*, we have had more success with *S. thermophilus*, so the manual focuses only on it. Since the yogurts may contain other bacterial species, students start by producing t-streaks of either commercial or heirloom yogurts to isolate colonies for screening. In our implementation of the class, we have students work in groups of four, with one pair of students focusing on the heirloom yogurt and one pair working on the commercial. Commercial yogurts are obtained from the grocery store. Because yogurt is semi-solid and difficult to streak out, we recommend that students make at least three streak plates from each type of yogurt.

In the module presented here, we rely on brute-force PCR screening (next section) of many colonies as the way to simultaneous identify whether an isolate is *S. thermophilus* and also amplify its CRISPR loci. An optional way to extend this portion of the project would be to add a few lab sessions where students first screen candidate colonies using conventional approaches to differential identification. In previous implementations of the course, we have had students microscopically examine cell appearance, arrangement, and Gram characteristics, and conduct fermentation tests (in Durham tubes, with lactose, mannitol, or sucrose) and temperature sensitivity tests (at 45 and 15°C) to help discriminate *S. thermophilus* from other common lactic acid bacteria. (After the microscopic examination, students re-streaked their candidates so they wouldn't run out of cells). While this introduces students to traditional methods of microbial identification with Bergey's Manuals (Bergey and Holt, 2000; Vos et al., 2009), we have often found that the tests can be inconclusive and many students don't identify *S. thermophilus* until they screen new colonies during PCR anyway. The physiological approach to identifying bacterial unknowns has been used extensively for many decades as an inquiry-driven approach to college microbiology lab courses, and has been thoroughly described elsewhere (Callery et al., 1980; Ziser, 1983; Deutch, 1994; Wagner and Stewart, 2000; Johnson and Case, 2019), so here we limit our description to the PCR screen.

Amplification of CRISPR Loci

Students carry out colony PCR with primers that bind to conserved sequences just outside of the CRISPR repeat/spacer array. Colony PCR avoids time-consuming genomic DNA extraction, and the suggested schedule (**Figure 1**) affords time

for a "back up day" if student PCRs fail in their first attempt. If a student's positive control works, but they don't get any bands from their colonies, these colonies may not be *S. thermophilus*, and they should try new colonies. If the positive or negative control fails, students should have a discussion with the instructor about what may have gone wrong, and they can either pick new colonies or retry the initial, if there is enough material left. (For the "back-up" day, since it is hard to predict how many students will need to set up a second PCR reaction, we recommend against pre-aliquoting reagents. Instead, we just keep a few stock tubes on hand for the groups that need them).

The forward primer, ST1_fwd, is 5′-TGCTGAGACAACCTAGTCTCTC-3′, and the reverse primer, ST1_rev, is 5′-TAAACAGAGCCTCCCTATCC-3′. These are the primers "yc70" and "CR1-rev" from Horvath et al. (2008), renamed here to avoid student confusion. Four different CRISPR loci have been detected in *S. thermophilus* genomes; these primers are specific for the CRISPR1 locus (Horvath et al., 2008; Barrangou et al., 2013). Unlike the other loci, which are completely absent in some *S. thermophilus* strains, the CRISPR1 locus appears to be ubiquitously present amongst *S. thermophilus* strains, while simultaneously harboring the most diversity in its individual spacer sequences from strain to strain (Horvath et al., 2008). A lab-grown culture of *S. thermophilus* (available to purchase from ACCT), serves as the positive control.

Students determine the size of their CRISPR amplicons through gel electrophoresis. Since instructional labs utilize varying manufacturers of electrophoresis chambers, power supplies, and gel imaging instruments, we have left the accompanying protocol for this section intentionally vague. As a safety consideration, we do suggest using SYBR safe and blue LED illumination instead of ethidium bromide and ultraviolet light; however, either option will work. Be sure to remind students to save their PCR products; this is what they will submit for sequencing in the next step.

Sequencing of CRISPR Loci

Once each group of students has a successful commercial and heirloom amplicon, they can submit them as templates for conventional Sanger sequencing. The ST1_fwd primer is used as a sequencing primer, and it is sufficiently upstream of the spacer array so that the start of the array can be accurately sequenced. Different sequencing service providers will have different specifications for the volume and concentration of template and primer. We use a service provider that will carry out PCR clean-up (removal of excess primers, protein, and dNTPs) for a small additional fee, but this could also be carried out by students if time allows.

It is important to note that the typical length of a Sanger sequencing run may not be sufficient to cover the entire amplified CRISPR array. In our implementation of the class, we have students focus on the 5′ end of the array, since this region reflects the most recent history of virus exposure (new spacers are added to the 5′ end of the array (Barrangou et al., 2007). However, if time and coincidence with course learning outcomes allows it, students could use a primer walking strategy to sequence the entire array, using their first sequencing results to design a forward sequencing primer corresponding to a spacer (not a repeat) about 100 bp from the 3′ end of their sequence.

Characterization of Phage-Derived Spacers

Once students have their sequences back, they will identify the individual spacer sequences. This can be done manually simply by looking for stretches of sequence that seem to be repeated, then flagging the variable regions in between. I have provided an in-class activity (**Supplementary Material**) where students are challenged to do that using two common lab strains of *S. thermophilus*. However, to ensure reproducibility and make things a little less tedious for the students, for the actual results from the commercial or heirloom yogurt we have students use a bioinformatics tool, CRISPRCasFinder, that will identify repeat regions and create a list of spacers automatically (Couvin et al., 2018, available as a web-based tool[1]).

The CRISPRCasFinder web tool requires sequences in the fasta format. Many Sanger sequencing services provide output in the '.seq' format. It is easy to convert the files in a text-editor (by adding a ">sequencename" header line), and there are also web tools like EMBOSS seqret[2] that can perform the conversion.

Once spacers are identified, students can use the NCBI's web-based BLAST to search for matches in its database[3]. It is important for students to pay attention to the organism names and focus on viral matches, as many of the top hits may actually be coming from the CRISPR arrays of closely related *S. thermophilus* strains. Some spacers may not return any viral matches; these are derived from new viruses whose sequence is not in the database.

Once students have a list of their spacers and a list of the amplicon lengths for the commercial and heirloom isolates, the intellectual work of interpreting the results comes in. Students can perform *t*-tests on the amplicon sizes (derived from the gel) to see if there is a statistically significant difference between commercial and heirloom samples in terms of the number of spacers. (Since spacers and repeats are regular lengths, the amplicon length is an effective proxy for the number of spacers). Students can also research the viruses their bacteria have immunity against to learn more about them: Where are they commonly found? What is known about their normal function in microbial ecosystems? Do they have any connection to the fermented food industry? Is there anything known about their potential role in human health? Sometimes there is not much information about a particular virus, but even looking up information on who submitted a virus genome (for example, a yogurt manufacturer), can provide insight. These research questions will require students to perform literature searches, read scientific journal articles, generate hypotheses and construct arguments for them with evidence. There are many possible ways students could demonstrate how they have researched these questions and how they interpret their results (lab reports,

[1]https://crisprcas.i2bc.paris-saclay.fr/CrisprCasFinder/Index

[2]https://www.ebi.ac.uk/Tools/sfc/emboss_seqret/

[3]https://blast.ncbi.nlm.nih.gov/Blast.cgi

short essay questions on a worksheet, posts in a discussion forum, group presentations). In our implementation of the module, we explicitly (via a rubric) directed students to explore specific research questions in the discussion section of lab reports (**Table 2**).

RESULTS

Implementation of Module in Undergraduate Lab Course

This module was incorporated into a redesigned upper-level Microbiology Laboratory course taken primarily by juniors and seniors. The module has been taught by three different instructors (including the author) since it was first developed in 2019, with eight offering of the class reaching ∼450 students (in person). In Spring 2020, two sessions of the class were held remotely for an additional ∼100 students, using a modified version of the module with analysis portion only, relying on data generated by previous classes.

Incorporating new activities into large-enrollment lab courses can be difficult, since there are often many different people involved. In our implementation of the course, there are multiple instructors teaching separate sections of the class every quarter. Multiple graduate instructional assistants (1 per 24 students) help lead the labs, and instructional laboratory support staff help prepare and aliquot reagents. To ensure that everyone was on the same page, the lead instructor for the course (the author) held weekly planning meetings with the entire instructional team. Additionally, a scale-up approach was taken to deploying the course — it was first piloted with a single instructor (again, the author) and small group of 24 students before expanding it to every section.

The module has been used to successfully gather data in every offering of the course. Sometimes, individual students may not be able to get PCR amplicons from their yogurt despite sampling multiple colonies; one possible reason is that *S. thermophilus* may have been present at particularly low-abundance in their sample. One potential way to improve the yield of *S. thermophilus* colonies would be to switch from MRS media to a more selective media, like M17 (Shankar and Davies, 1977). In cases where individual students are unable to obtain an amplicon, we have been able to redistribute amplicons from groups that had multiple successful PCR bands. In CURE modules that incorporate open-ended research, it is important to allow for the possibility of failure, and have back up material for analysis. We make sure not to look for specific or expected results as part of our graded assessments — instead we focus on student's understanding of how results were generated and their ability to interpret them and make scientific arguments. When we do want to assess technical skills, we do so with small practical exams not tied to the main research module.

TABLE 2 | Guidelines for student exploration of data.

	Guidance used for student exploration and interpretation of data
1	Interpret 16S data in terms of how heirloom different from commercial and any unexpected results
2	Interpret meaning of class-wide results for difference in the CRISPR array size in commercial vs heirloom
3	Speculate on whether results do or do not have implications for human health (discuss general implications from class data as a whole AND at least one specific implication of one of the particular viruses your strains are immune to)
4	Discuss limitations of this project and ways to improve the method
5	Suggest future experimental or literature or database research directions

To earn full credit, students needed to include all items in the discussion section of their lab reports. (Full lab report rubric available in ***Supplementary Material****).*

What Was Learned About the CRISPR Loci in Yogurt-Derived *S. thermophilus* So Far?

Data collection and analysis is ongoing, but the size of CRISPR1, as measured by the amplicon size, varies considerably, from as small as 0.8 up to 4.0 kb. The loci tend to be bigger in the commercially derived strains, however, students do not always find that this difference is statistically significant. This may be due in part to errors in estimating band size — students estimate it "by eye" using DNA ladders. In the future we will have students fit a trendline to the distance traveled by each band in that ladder, and use that and the distance of their amplicon to more precisely calculate the size. Alternatively, primer-walking or a long-reads approach like nanopore sequencing could be used to sequence the entire array and more precisely measure its length from first to last spacer. Another potential factor is that students only used the amplicon size data generated by the other groups in their section (∼48 students, or 12 groups); and it is possible this is too small a sample to see significance. A third possibility is that a key difference between commercial and heirloom yogurts may not be in the number of phage they are resistant to, but in the phylogenetic diversity of those phage, which is another item students could explore. Going forward, we would like to incorporate more systematic analysis of the accumulated data from successive offerings of the course; this will also allow students to get a better sense of how science is an iterative process.

What Did Students Learn From Participating in This Module?

An exploratory assessment of student knowledge and attitudes before and after completing the CRISPR module was conducted during one quarter of the course. In a brief pre- and post-module survey, students were asked to rate their agreement with several statements about yogurt, probiotics, the human gut microbiome, and their attitudes toward commercially produced and genetically modified food (shown in **Table 3**). Some items were based on an instrument used to assess healthcare professionals' conceptions of probiotics (Wilson and Whitehead, 2019).

Quantitative analysis of the survey data showed that, for most items, there was no significant change in student response (**Table 3**). Among items which did show a significant shift in student response, items 5 and 6, "Bacteria are used to produce yogurt" and "All yogurt sold in stores contains bacteria" show an increase in students correct understanding of the role microbes

TABLE 3 | Student agreement with statements from exploratory survey.

Statement *responses were strongly disagree (0), disagree (1), neutral (2), agree (3), and strongly agree (4)*	Pre-score		Post-score			
	Mean	*SD*	*Mean*	*SD*	*Wilcoxon W*	*P*
1. Eating foods or supplements with certain types of bacteria can have a positive impact on human health.	3.15	0.795	3.27	0.761	24.00	0.430
2. In order to have any impact on human health, microbes must be alive at the time they are consumed.	2.45	1.063	2.85	1.093	49.00	0.055
3. In order to have any impact on human health, microbes must stay alive for at least part of the time they spend in the digestive system.	2.70	0.847	2.73	1.039	88.50	0.909
4. Commercially produced yogurt is safer than homemade yogurt.	2.15	0.906	2.12	1.083	133.50	0.825
5. Bacteria are used to produce yogurt.	3.33	0.777	3.88	0.331	8.50	< 0.001*
6. All yogurt sold in stores contains bacteria.	2.73	1.098	3.48	0.906	8.00	< 0.001*
7. Some yogurt sold in stores contains living bacteria.	3.18	0.0.584	2.91	1.208	135.00	0.251
8. All yogurt sold in stores contains living bacteria.	1.94	1.029	2.82	1.211	60.00	0.002*
9. We use this statement to discard the surveys of people who are not reading the questions. Please select "Agree" for this question to preserve your answers.						
10. I am comfortable eating food developed in a (food-grade) laboratory.	3.06	0.747	3.30	0.728	26.50	0.084
11. I am comfortable eating genetically modified foods.	3.03	0.770	3.18	0.769	28.00	0.178
12. Natural foods are safer than commercially produced foods.	2.45	0.833	1.97	0.728	170.00	0.011
13. Scientists have a good understanding of how the human gut microbiome influences health.	2.30	0.984	2.06	1.144	107.50	0.334

Deidentified student response data was analyzed as follows. Only students who completed both the pre- and post-survey on time were included, and students who did not respond to all items were removed. Students who did not correctly respond to the control statement (item 9) were removed, and this item was not used in subsequent analysis. For each item, students selected "Strongly Disagree," "Disagree," "Neutral," "Agree," or "Strongly Agree;" these were converted to scores of 0, 1, 2, 3, or 4, respectively. Pre- and post- scores for each student were compared using a Wilcoxon signed-rank test (non-parametric, paired, n = 33). Statistics were computed in jamovi (The jamovi project, 2020). Mean scores and standard deviation are shown, and items with a significant difference in response distribution (Bonferroni corrected α *of* $p < 0.004$*) are starred (*).*

play in the everyday production of food in the real world. There was also a significant increase in item 8, "All yogurt sold in stores contains living bacteria," though this item perhaps reflects student perception, rather than underlying knowledge, since some yogurt is heat-treated to kill off the bacteria prior to sale.

Students were also asked to respond to the open-ended question "What was the most important thing you learned from the microbes and industry (yogurt) module?" Selected responses are shown in **Table 4**. Some students (student 1 and student 2 in the table) reported that it was easier to understand CRISPR when they see where it comes from, despite having learned about its use in genetic engineering in their other courses. This suggests that incorporating the basic microbiology of modern applied techniques may help students better understand them. Other students (student 3 and student 4 in the table) emphasized that this module gave them an increased awareness of microbiology's role in their everyday lives. (All responses were collected with approval of UC San Diego Institutional Review Board).

It is likely that there are other underlying gains in student understanding and attitudes toward science not addressed by this exploratory survey. Future assessments of the impact of this module on content understanding and attitudes will feature items more specifically connected to the module, and will use Likert scales with multiple items to assess each content area to improve accuracy.

Ideally, our assessment would measure broader impacts of the module on student understanding of the nature of science and the psychosocial attitudes associated with persistence in science. The extent to which students perceive dimensions of research could be measured using instruments such as the Laboratory Course Assessment Survey (Corwin et al., 2015). Student understanding of the nature of science could be measured with an instrument like the Student Understanding of Science and Scientific Inquiry (SUSSI) survey (Liang et al., 2008), and psychosocial outcomes could be measured with instruments like Persistence in the Sciences (PITS) Survey (Hanauer et al., 2016). This module was implemented as part of a full redevelopment of our microbiology lab curriculum, which included other substantial changes and a second CURE module in addition to this module. When comparing the revised version of the course to previous versions, it would be impossible to distinguish the effects of the CRISPR module from effects caused by the other changes. We hope that future assessments of this module can include these broader outcomes, with a particular focus on whether the module has a positive impact on students from underrepresented groups.

DISCUSSION

This article shares a module that could be deployed to bring more research into existing classes. The module asks a research question with potential bearing on human health, and the research question is ideally suited for a CURE, in that it benefits from collection and analyses of many data points that would be difficult to automate. Here, broad lessons learned from our initial offerings of the course, as well as potential future directions, will be discussed.

TABLE 4 | Selected student responses to open-ended question on what they learned from module.

	What was the most important thing you learned from the microbes and industry (yogurt) module?
Student 1	*"I had a much better understanding on how CRISPR arrays works and how CRISPR is prevalent in common things and not just in a lab. if that makes sense."*
Student 2	*"The most important thing I learned was the mechanism of CRISPR, as I had learned about CRISPR in other science classes but never learned where it came from and exactly how it works, which could be crucial as it appears the age of genetic modification is inevitable."*
Student 3	*"How we can analyze the CRISPR loci to determine viruses that have infected yogurt bacteria and how this might give insight to how it affects our health and which kind of yogurt we consume."*
Student 4	*"That commercial yogurt is 'vaccinated' against a wide variety of phages in order to resist more attacks."*

Lessons Learned

We had students share data with instructors and each other through shared, editable, online spreadsheets (in google docs). They then drew on this data when writing up lab reports. A drawback of this was that students didn't always supply all of the requested information, and the data sheets experienced a kind of "format creep" as edits were sometimes made to the underlying structure, making it difficult to swiftly extract specific fields for later analysis. Going forward, a more robust data collection method will be used, perhaps making use of online-forms with questions that must be answered in order to earn full credit on assessments. Not only will this help ensure data is reported correctly, it should make it easier to assemble and compare data from multiple classes.

Another lesson was that it was difficult, as a class, to synthesize everyone's data for a holistic analysis. In lab reports, students focused mostly on their own results, especially for the viral matches to the CRISPR spacers in their strain. In the future, there will be more time built into the class itself for discussion of the overall results. Even then, it may be hard to systematically summarize all of the data generated. Since one of the goals of the CURE is to contribute to science, this summative analysis is an important component. In the future, graduate student researchers may be recruited to help with this analysis, or it could be used as the basis of a data-focused undergraduate seminar course.

Even if additional analysis by other students is required, we want to make sure that every student participating in the module has a sense of agency while analyzing results. So far, we tried to accomplish that mainly through student exploration of research questions specific to their CRISPR loci (**Table 2**), as large class sizes limit the number of alternative materials we could provide for students if we asked them to design their own experiments. There are however, opportunities to incorporate more student agency. Students could choose the species and/or particular CRISPR loci (if a species has more than one), and design their own primers to amplify that CRISPR region. Without changing the wet-lab protocol, it could also be possible to give students more agency with a less prescriptive final assessment — rather than strictly following a lab report rubric, students could propose and pursue a literature and/or bioinformatic research question of their own choosing, which could increase their sense of project ownership.

Online Options

In Spring 2020, with the global pandemic forcing remote instruction, students carried out the analysis steps with data from a previous quarter. In retrospect, this may have been a missed opportunity, as NCBI's microbial genomes[4] currently has genomes for 70 different *S. thermophilus* strains. Students could copy the CRISPR1 locus (or make a virtual amplicon based on the primers) and then characterize the CRISPRs of these strains. Students could even characterize other CRISPR loci in *S. thermophilus* or the CRISPR loci of other lactic acid bacteria involved in fermentation. Carrying out the analysis on public data sets is a viable option for labs forced online, or for instructors wishing to incorporate this module into a lecture course or a dry-lab (bioinformatics) course.

Future Directions

There is a certain tension inherent in sharing a specific CURE module with the educational community. The goal is to get more students involved in research and to recruit contributors of additional data, so we aim to show that has been successful. However, for the CURE to be a genuine research opportunity, the scientific outcome of the research question can't already be solved, and indeed, to make the CURE sustainable over a period of more than a few years, should be something open to continuous addition and refinement. This CURE certainly has not yet fully resolved its central questions of what immunity is present in strains used for food production and what the potential consequences of that immunity are for human health. The first goal is to create a catalog of CRISPR spacers found in *S. thermophilus* used in yogurt production; the listings in this catalog could be expanded and validated by additional contributions, and could be broadened to include other species and other types of fermented food. Ultimately this catalog could be used a resource to better understand potential interactions of microbes we consume with the human gut microbiome. To that end, the plan is to create an online public repository for this data. Queries from instructors and researchers interested in participating are welcome.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by UC San Diego Human Research Protections Program Institutional Review Board (opt-out consent forms were distributed in accordance with approved research plan; participants who opted out were removed and data was de-identified by the university before analysis began). Written

[4]https://www.ncbi.nlm.nih.gov/genome/microbes/

informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

KP developed this course module, wrote instructional materials for it, collected and analyzed assessment data, and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.578737/full#supplementary-material

Supplementary Data Sheet 1 | CRISPR-finding by hand activity.

Supplementary Data Sheet 2 | CRISPR-finding by hand activity answer key.

Supplementary Data Sheet 3 | Lab Manual w. Background and Protocols.

Supplementary Data Sheet 4 | Lab report rubric.

REFERENCES

American Association for the Advancement of Science [AAAS] (2011). *Vision and Change in Undergraduate Biology Education*. Washington, DC: American Association for the Advancement of Science.

Aryana, K. J., and Olson, D. W. (2017). A 100-year review: yogurt and other cultured dairy products. *J. Dairy Sci.* 100, 9987–10013. doi: 10.3168/jds.2017-12981

Auchincloss, L. C., Laursen, S. L., Branchaw, J. L., Eagan, K., Graham, M., Hanauer, D. I., et al. (2014). Assessment of course-based undergraduate research experiences: a meeting report. *CBE Life Sci. Educ.* 13, 29–40. doi: 10.1187/cbe.14-01-0004

Bangera, G., and Brownell, S. E. (2017). Course-based undergraduate research experiences can make scientific research more inclusive. *CBE Life Sci. Educ.* 13, 602–606. doi: 10.1187/cbe.14-06-0099

Barr, J. J. (2017). A bacteriophages journey through the human body. *Immunol. Rev.* 279, 106–122. doi: 10.1111/imr.12565

Barr, J. J., Auro, R., Furlan, M., Whiteson, K. L., Erb, M. L., Pogliano, J., et al. (2013). Bacteriophage adhering to mucus provide a non–host-derived immunity. *Proc. Natl. Acad. Sci. U.S.A.* 110, 10771–10776. doi: 10.1073/pnas.1305923110

Barrangou, R., Coûté-Monvoisin, A. C., Stahl, B., Chavichvily, I., Damange, F., Romero, D. A., et al. (2013). Genomic impact of CRISPR immunization against bacteriophages. *Biochem. Soc. Trans.* 41, 1383–1391. doi: 10.1042/BST20130160

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., et al. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. *Science* 315, 1709–1712. doi: 10.1126/science.1138140

Barrangou, R., and Horvath, P. (2017). A decade of discovery: CRISPR functions and applications. *Nat. Microbiol.* 2:17092. doi: 10.1038/nmicrobiol.2017.92

Bergey, D. H., and Holt, J. G. (2000). *Bergey's Manual of Determinative Bacteriology 1860-1937*. Philadelphia, PA: Lippincott Williams & Wilkins.

Brownell, S. E., Hekmat-Scafe, D. S., Singla, V., Seawell, P. C., Conklin Imam, J. F., Eddy, S. L., et al. (2017). A high-enrollment course-based undergraduate research experience improves student conceptions of scientific thinking and ability to interpret data. *CBE Life Sci. Educ.* 14:ar21. doi: 10.1187/cbe.14-05-0092

Callery, M. L., Koritz, H. G., and Zipf, M. E. (1980). Microbiology in introductory biology. *Am. Biol. Teach.* 42, 416–421. doi: 10.2307/4447018

Cooper, K. M., Blattman, J. N., Hendrix, T., and Brownell, S. E. (2019). the impact of broadly relevant novel discoveries on student project ownership in a traditional lab course turned CURE. *CBE Life Sci. Educ.* 18:ar57. doi: 10.1187/cbe.19-06-0113

Corwin, L. A., Runyon, C., Robinson, A., and Dolan, E. L. (2015). The laboratory course assessment survey: a tool to measure three dimensions of research-course design. *CBE Life Sci. Educ.* 14:ar37. doi: 10.1187/cbe.15-03-0073

Corwin, L. A., Runyon, C. R., Ghanem, E., Sandy, M., Clark, G., Palmer, G. C., et al. (2018). Effects of discovery, iteration, and collaboration in laboratory courses on undergraduates' research career intentions fully mediated by student ownership. *CBE Life Sci. Educ.* 17:ar20. doi: 10.1187/cbe.17-07-0141

Couvin, D., Bernheim, A., Toffano-Nioche, C., Touchon, M., Michalik, J., Néron, B., et al. (2018). CRISPRCasFinder, an update of CRISRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. *Nucleic Acids Res.* 46, W246–W251. doi: 10.1093/nar/gky425

Cromley, J., Perez, T., and Kaplan, A. (2016). Undergraduate STEM Achievement and retention: cognitive, motivational, and institutional factors and solutions. *Policy Insights Behav. Brain Sci.* 3, 4–11. doi: 10.1177/2372732215622648

Cultures for Health (2020). Available online at: https://www.culturesforhealth.com/ (accessed June 30, 2020).

Deutch, C. E. (1994). Restructuring a general microbiology laboratory into an investigative experience. *Am. Biol. Teach.* 56, 294–296. doi: 10.2307/4449824

Fisberg, M., and Machado, R. (2015). History of yogurt and current patterns of consumption. *Nutr. Rev.* 73, 4–7. doi: 10.1093/nutrit/nuv020

Godde, J. S., and Bickerton, A. (2006). The repetitive DNA elements called CRISPRs and their associated genes: evidence of horizontal transfer among prokaryotes. *J. Mol. Evol.* 62, 718–729. doi: 10.1007/s00239-005-0223-z

Grens, K. (2014). *There's CRISPRs in Your Yogurt.* The Scientist Magazine. December 31st, 2014.

Hanauer, D. I., Graham, M. J., and Hatfull, G. F. (2016). A measure of college student persistence in the sciences (PITS). *CBE Life Sci. Educ.* 15:ar54. doi: 10.1187/cbe.15-09-0185

Hanauer, D. I., Jacobs-Sera, D., Pedulla, M. L., Cresawn, S. G., Hendrix, R. W., and Hatfull, G. F. (2006). Teaching scientific inquiry. *Science* 314, 1880–1881.

Horvath, P., Coûté-Monvoisina, A., Romero, D. A., Boyavala, P., Fremaux, C., and Barrangou, R. (2009). Comparative analysis of CRISPR loci in lactic acid bacteria genomes. *Int. J. Food Microbiol.* 131, 62–70. doi: 10.1016/j.ijfoodmicro.2008.05.030

Horvath, P., Romero, D. A., Coûté-Monvoisin, A., Richards, M., Deveau, H., Moineau, S., et al. (2008). Diversity, activity, and evolution of CRISPR Loci in *Streptococcus thermophilus*. *J. Bacteriol.* 190, 1401–1412. doi: 10.1128/JB.01415-07

Hurtado, S., Newman, C. B., Tran, M. C., and Chang, M. J. (2010). Improving the rate of success for underrepresented racial minorities in STEM fields: insights from a national project. *New Dir. Inst. Res.* 148, 5–15. doi: 10.1002/ir.357

Ishino, Y., Krupovic, M., and Forterre, P. (2018). History of CRISPR-Cas from encounter with a mysterious repeated sequence to genome editing technology. *J. Bacteriol.* 200:e00580-17. doi: 10.1128/JB.00580-17

Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., and Nakata, A. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. *J. Bacteriol.* 169, 5429–5433. doi: 10.1128/jb.169.12.5429-5433.1987

Johnson, T. R., and Case, C. L. (2019). *Laboratory Experiments in Microbiology*, 12th Edn. London: Pearson.

Lander, E. S. (2016). The heroes of CRISPR. *Cell* 104, 18–28. doi: 10.1016/j.cell.2015.12.041

Liang, L. L., Chen, S., Chen, X., Kaya, O. N., Adams, A. D., Macklin, M., et al. (2008). Assessing preservice elementary teachers' views on the nature of scientific knowledge: a dual-response instrument. *Asia Pacific Forum Sci. Learn. Teach.* 9:1.

Mojica, F. J., Díez-Villaseñor, C., García-Martínez, J., and Soria, E. (2005). Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J. Mol. Evol.* 60, 174–182. doi: 10.1007/s00239-004-0046-3

Ogilvie, L. A., and Jones, B. V. (2015). The human gut virome: a multifaceted majority. *Front. Microbiol* 6:918. doi: 10.3389/fmicb.2015.00918

Pourcel, C., Salvignol, G., and Vergnaud, G. (2005). CRISPR elements in *Yersinia pestis* acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. *Microbiology* 151, 653–663. doi: 10.1099/mic.0.27437-0

Rowland, S., Pedwell, R., Lawrie, G., Lovie-Toon, J., and Hung, Y. (2017). Do we need to design course-based undergraduate research experiences for authenticity? *CBE Life Sci. Educ.* 15:ar79. doi: 10.1187/cbe.16-02-0102

Russell, C. B., and Weaver, G. C. (2011). Chemistry education research and practice a comparative study of traditional, inquiry-based, and research-based laboratory curricula: impacts on understanding of the nature of science. *Chem. Educ. Res. Pract.* 12, 57–67. doi: 10.1039/C1RP90008K

Shankar, P. A., and Davies, F. L. (1977). A note on the suppression of *Lactobacillus bulgaricus* in media containing β-glycerophosphate and application of such media to selective isolation of *Streptococcus thermophilus* from yoghurt. *J. Soc. Dairy Technol.* 30:28. doi: 10.1111/j.1471-0307.1977.tb01162.x

Shkoporov, A. N., Clooney, A. G., Sutton, T. D. S., Ryan, F. J., Daly, K. M., Nolan, J. A., et al. (2019). The human gut virome is highly diverse, stable, and individual specific. *Cell Host Microbe* 26, 527–541. doi: 10.1016/j.chom.2019.09.009

The jamovi project (2020). *jamovi*. (Version 1.2) [Computer Software].

Veiga, P., Pons, N., Agrawal, A., Oozeer, R., Guyonnet, D., Brazeilles, R., et al. (2015). Changes of the human gut microbiome induced by a fermented milk product. *Sci. Rep.* 4:6328. doi: 10.1038/srep06328

Vos, P., Garrity, G., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., et al. (2009). *Bergey's Manual of Systematic Bacteriology: The Firmicutes*. New York: Springer, 3.

Wagner, S. C., and Stewart, R. S. Jr. (2000). Microbial safari: isolation & characterization of unknowns in an introductory microbiology laboratory. *Am. Biol. Teach.* 62, 588–592. doi: 10.2307/4450983

Wilson, Z., and Whitehead, K. (2019). A cross sectional survey to assess healthcare professionals' attitudes to and understanding of probiotics. *Clin. Nutr. ESPEN* 34, 104–109. doi: 10.1016/j.clnesp.2019.08.004

Ziser, S. (1983). The identification of unknown bacteria. *Iowa Sci. Teach. J.* 20:5.

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ORIGINAL RESEARCH
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Tackling Real-World Environmental Paper Pollution: A Problem-Based Microbiology Lesson About Carbon Assimilation

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Governmental and educational organizations advocate for the adoption of inquiry-based, student-centered educational strategies in undergraduate STEM curricula. These strategies are known to benefit students by increasing performance, enhancing mastery of class content, and augmenting affect, particularly in underrepresented racial/ethnic minority students. Among these strategies, case study and project-based learning allow students to master course content while collectively tackling relevant, real-world societal problems. In particular, environmental pollution with paper-based products provide a current problem by which microbiology students learn about the role of microorganisms in paper waste management as well as the microbiological and biochemical processes involved in protein secretion, nutrient uptake, and energy metabolism. Delivered in a flipped, hybrid class in a Technology-Enabled Active Learning (TEAL) laboratory, this lesson taught students about exoenzyme secretion, biopolymer hydrolysis, intracellular transport of sugars, and sugar catabolic reactions. Students demonstrated increased comprehension of exoenzyme function and secretion, as well as how cells uptake the products of exoenzyme hydrolysis. However, students had challenges in placing the transported exoenzyme products within metabolic processes. Our results show increased perceived learning from the students as well as an understanding of the societal implications of these microbiological concepts. Our lesson deviated from knowledge silos in which students learn information in discrete topics. While departing from employing traditional, compartmentalized learning approaches, this student-centered guided lesson frames the systemic nature of the microbiological and biochemical processes underlying the decomposition of organic matter in a real-world context.

Keywords: paper pollution, 5E model, problem-based learning, microbiology, pedagogy

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INTRODUCTION

Institutions and faculty are revolutionizing their science, technology, engineering, and mathematics (STEM) educational programs to effectively engage and train tomorrow's scientists (AAAS, 2009). Students need more than a traditional biology education to tackle the most pressing issues facing science and society; they need to learn how to address real-world problems. By presenting a problem

in an experiential learning environment, students can build their own conceptual framework through an active learning process that encourages them to socially engage, share ideas, and participate in their own inquiry-based learning (Allen and Tanner, 2005; Chorazy and Klinedinst, 2019). Problem-based learning fosters deep, accurate understanding of a subject and contributes to developing process skills such as research, teamwork, and verbal communication (Allen et al., 2011; Orsmond and Zvauya, 2015). Problem-based learning increases awareness and connects students to local challenges, which is instrumental in actively engaging them in their education, especially for underrepresented student populations (Nelson-Hurwitz and Buchthal, 2019).

When students are passionate about a subject, they engage deeply in their learning (Harackiewicz et al., 2016), and one issue that students are passionate about is the environment (Desa et al., 2011). A growing global crisis is waste pollution, and contrary to many perspectives, paper is a major contributing factor to global waste. The average American consumes 700 pounds of paper each year and paper represents 25% of waste in landfills and 33% of municipal waste (Desilver, 2016). Now more than ever, it is important to foster students who can critically assess these issues by understanding how natural biodegradation cycles operate to ameliorate stress on our global ecosystem (Marope, 2016). The use of deliberate pedagogy can increase awareness of paper waste pollution and emphasize how microbial degradation provides an alternative to paper waste management (Nelson-Hurwitz and Buchthal, 2019).

In this paper, we test the use of evidence-based teaching practices in combination with problem-based learning in a flipped classroom to ask if these have a positive impact on students' understanding of complex biological material in the context of a real-world problem. Specifically, we wanted to ascertain if the activity would increase student's mastery of the concepts of bacterial protein secretion, nutrient transport, and carbon cycle. Furthermore, we wanted to valuate if the activity would influence the student's confidence about their knowledge of these topics. Here we present an effective lesson that utilizes paper waste degradation as a platform to teach students about the carbon cycle. Our work shows that this lesson increases student's awareness of paper waste pollution and management, content knowledge about microbial paper degradation, and mastery of the process of bacterial protein secretion, nutrient transport and metabolism.

Pedagogical Framework and Principles

The Technology-Enabled Active Learning (TEAL) Laboratory Environment

The General Microbiology course at the University of California, Merced (UC Merced) is a hybrid class taught using flipped pedagogy, and it is delivered in a TEAL laboratory environment. The TEAL lab is a classroom space designed to offer students the opportunity to enhance their cognitive and behavioral engagement through small group discussions, peer evaluation, and shared experiential learning. Students interact more with each other, share resources, and experience a more equitable learning environment in the TEAL lab (Beichner et al., 2007). These labs facilitate the implementation of active learning strategies to best utilize the classroom space (Cotner et al., 2013).

The General Microbiology TEAL lab houses 90 students in ten working tables (**Supplementary Figure 1**). These are arranged to allow equal view of two large class projector screens located in the front and back of the room. Each table is equipped with docks to power laptops, a document camera to display paper-based work, a whiteboard, an HDMI monitor and a control panel. The instructor control lectern is centered in the room and has the ability to orchestrate the technology offered in the space. In this way, class content can stream from the lectern, or from any of the ten working tables to the entire class (Office of Information Technology, UC Merced, 2020).

Active Learning and Flipped Hybrid Classrooms

Active learning is a student-centered pedagogy where students interact with their learning as opposed to passively listening to a lecture. It is widely accepted for its efficacy in increasing students' performance, especially in STEM courses (Freeman et al., 2014). In order to use the space and time with the students most effectively, we developed a flipped classroom model designed to center the class around the students through facilitated experiential learning. Students watch class content videos at home and attend their scheduled lecture hour prepared for activities with a heightened sense of engagement (Gilboy et al., 2015). The hybrid flipped classroom provides students with a more intimate experience with their instructors, where they can benefit from team-based learning, demonstrate their knowledge, and receive immediate feedback.

To amplify the effects of an active team-based learning environment, our lesson structure follows a recommended instructional protocol by Michaelsen and Sweet (2011). Students begin class with readiness assurance practices, where they (1) watch video lectures before class; (2) respond individually to a 10-min lecture comprehension quiz at the start of lecture (**Supplementary Material 2**); (3) review the quiz as a table to confirm answers; and (4) discuss the quiz results as a class to identify and diffuse misconceptions and establish consensus. The readiness assurance is followed by a 55-min flipped lecture, which consists of a brief review of material followed by an activity based on the 5E model.

The 5E Model: Engaging Large Classes

To address the needs of a large active-learning class, it is necessary to implement a well-documented form of learning that focuses on the desired learning outcomes and considers the cognitive engagement of the students. The learning-cycle method known as the "5E model" (Bybee, 1997) is a common method for organizing large biology-based lessons infused with active learning (Allen and Tanner, 2005). The 5E model, comprised of five stages (engage, explore, explain, elaborate, and evaluate), is known

Abbreviations: ERI, emergency remote instruction; LMS, learning management system; PTS, phosphotransferase system; sec system, secretion system; STEM, science, technology, engineering and mathematics; tat system, twin-arginine translocation system; TEAL, technology enabled active learning.

for its efficiency and positive effects on students' broad-scale academic achievement, attitudes toward lessons, and science process skills (Cakır, 2017).

In order to bring the 5E framework into the context of our microbiology lesson, we integrated a constructivist approach to help students assemble their own knowledge and build a cooperative teamwork dynamic (von Glasersfeld, 1987; AAAS, 2009; Arik and Yilmaz, 2020). We used backward design to create class materials, first defining the learning outcomes and then charting steps to reach the desired level of understanding (Wiggins and McTighe, 1998). We developed activities, discussions and questions that would help students accomplish those outcomes. We integrated a team-based learning experience, proven to foster authentic perspectives to complex problems beyond the scale of individual learning (Michaelsen and Sweet, 2011). This established team-based approach, together with active-learning, helps underrepresented students succeed in STEM courses (Freeman et al., 2014; Snyder et al., 2016). This is particularly important at UC Merced, a Hispanic Serving Institution with over 70% first generation students.

MATERIALS AND METHODS

Research Setting

The University of California, Merced

UC Merced is the first research university built in the 21st century in the United States and serves the predominantly underserved communities of California's San Joaquin Valley. UC Merced holds a diverse student population, with 87% of students from traditionally underrepresented groups. The university has over 8,800 students and is designated as both a Hispanic Serving Institution (HSI) and an Asian American/Native American/Pacific Islander Serving Institution (AANAPISI).

Participants

Our student population included 89 students in the Fall 2019 course and 90 students in the Spring 2020 course (n = 179). The predominant racial and ethnic make-up of the combined cohorts consisted of 34.1% Asian and 33.5% Hispanic/Latinx, reflecting the University's HSI and AANAPISI designations. The remaining student population in the course was comprised of 2.8% American Indian/Alaska Native, 5% Black, 3.4% Native Hawaiian/Pacific Islander and 15.6% White. The cohort is primarily comprised of female students (65.4%). The mean age is 21 years of age. The primary difference between the cohorts is class level; the Fall 2019 class was mostly Senior students (93%) while the Spring 2020 cohort had 31% Juniors and 69% Seniors. Students GPA did not differ significantly between the two cohorts [$t(177) = -1.22$, $p = 0.22$], being 2.89 for Fall 2019 and 2.96 for the Spring 2020 (**Table 1**).

TABLE 1 | Student participant demographics.

	Fall 2019		Spring 2020		Overall	
	n = 89		*n* = 90		*n* = 179	
	n	(%)	*n*	(%)	*n*	(%)
Gender						
Male	26	(29.2)	36	(40.0)	62	(34.6)
Female	63	(70.8)	54	(60.0)	117	(65.4)
Race/ethnicity						
American Indian/Alaska Native	2	(2.2)	3	(3.3)	5	(2.8)
Asian	28	(31.5)	33	(36.7)	61	(34.1)
Black	5	(5.6)	4	(4.4)	9	(5.0)
Hispanic/Latinx	32	(36.0)	28	(31.1)	60	(33.5)
NH/PI[1]	3	(3.4)	3	(3.3)	6	(3.4)
White	14	(15.7)	14	(15.6)	28	(15.6)
Two or more races	3	(3.4)	4	(4.4)	7	(3.9)
Declined[2]	2	(2.2)	1	(1.1)	3	(1.7)
Age — mean (range)	21.3 (18–23)		21.3 (20–27)			
Class — (%)						
Junior		(7)		(31)		
Senior		(93)		(69)		
GPA[3] — mean ± *SD*	2.89 ± 0.38		2.96 ± 0.42		2.92 ± 0.41	

[1]NH/PI, Native Hawaiian/Pacific Islander. [2]Students who declined to report race/ethnicity. [3]Grade Point Average.

Format of General Microbiology at UC Merced

General Microbiology (BIO120) is an upper division course taken primarily by biology majors, and it is a graduation requirement for students in the Microbiology and Immunology emphasis track. The course requirements include General Biology, Molecular Biology, and Cellular Biology. The most relevant content to General Microbiology that students learn in these pre-requisite courses includes the overall organization and structure of cells, principles of metabolism and nutrient cycles, as well as the function of enzymes.

General Microbiology meets twice a week for 75 min in the TEAL lab, limiting the class size to 90 learners. The teaching team includes the instructor of record (García-Ojeda), one co-instructor (Shay), and one undergraduate learning assistant (Solis). This course was transformed into a flipped, team-based learning class in Fall 2015 and converted into a hybrid course in Fall 2019. The hybrid model used in this class consist of in-person flipped lectures with online discussion sections (**Supplementary Material 5**). The academic year 2019–2020 was the first full year to incorporate all learning and teaching techniques discussed in this manuscript.

Learning Outcomes

We chose the global issue of environmental pollution by paper-based products to teach students about the role of microorganisms in paper waste management. At this point in the course, students have been introduced to the history and fundamentals of microbiology, the structures and organization of microbial cells, the evolution and diversity of microbes, microbial motility, microbial growth, nutrient transport, and the nitrogen cycle. This section of the class focuses on carbon acquisition as well as how microbes secrete exoenzymes to hydrolyze macromolecules. Specifically, students learn about

the process of bacterial protein secretion, and how different exoenzymes break down lipids, nucleic acids, proteins, and carbohydrates. Students are then asked to connect the process of active nutrient transport via symporters, ABC transporters, or phosphoenolpyruvate-carbohydrate phosphotransferase system (PTS). Lastly, students demonstrate how the transported molecules are further modified by converting enzymes and then metabolized.

General Microbiology Course Learning Outcomes

The General Microbiology Course Learning Outcomes were designed to integrate previous learning and develop advanced scientific skills such as research and critical thinking. The following course learning outcomes related to this activity are:

(1) Recognize microbiological concepts and terms used in the primary scientific literature and to communicate with other microbiologists and scientists.
(2) Discern the molecular, metabolic, structural, and ecological differences between microbial cells and be able to explain how these differences allow microbes to (a) live in almost any environment on earth, (b) sense, react, and interact with their environment as well as with other organisms, and (c) serve as tools for science, medicine, and industry.
(3) Synthesize knowledge gained in previous courses and apply it to novel microbiological questions.

Carbon Acquisition Lesson Learning Outcomes

This lesson has the following learning outcomes:

(1) Given the biotic and abiotic sources of carbon and carbon-containing compounds, illustrate the biological flow of carbon, starting from an initial, complex carbon-containing molecule to a final product (CO_2 or fermentation product).
(2) Illustrate the enzymatic reactions that hydrolyze carbohydrates, nucleic acids, lipids, and proteins, and identify the various exoenzymes involved in this process.
(3) Identify how different microbes secrete important proteins.

For this lesson, we focused on the first two outcomes by asking students to work with a complex carbon-containing product: paper. Students work through the process of carbon flow, from the initial biomolecule carbon source in paper (cellulose) to the final metabolic product (CO_2 or a fermentation product) within Gram-negative bacterial cells. Students build an understanding of the overall carbon cycle and how biomolecules are hydrolyzed by exoenzymes secreted by bacteria. Exoenzyme secretion reinforces learning outcome 3, where students learn about the Sec and Tat translocators as well as the Type V secretion system and related proteins. Students also connect this lesson to previously taught material concerning the transport of monomeric sugar molecules across the cell membrane via PTS transporters as well as the catabolic reactions needed to extract energy under aerobic or anaerobic conditions (glycolysis, Entner-Doudoroff Pathway and Krebs cycle). Lastly, this lesson reinforces previously learned concepts of bacterial cell wall structure, proteins, cell membrane and their functions.

Pedagogical Format

Combining Strategies: Carbon Assimilation Group Exercise Using the 5E Model

The novelty of this pedagogical approach is the combination of strategies which seamlessly merge our highly active and collaborative learning initiatives. For this particular 75-min flipped lesson, students prepare by watching online videos about the carbon cycle, protein secretion systems, exoenzymes, and converting enzymes (for slides of this lecture, see **Supplementary Material 1.1**). They are also encouraged to listen to a podcast titled "How much of our stuff actually gets recycled?" (Brand, 2018).

A detailed summary of the lesson's timeframe can be found in **Supplementary Table 1**. For the *engage* phase of the 5E model, students spend the first 8 min of class taking a readiness assurance quiz (**Supplementary Material 2**). The quiz is followed by a short discussion of the lesson's learning outcomes (2 min) and a 5-min in-class lecture where students are introduced to current recycling challenges resulting from the adoption of *National Sword*, a 2018 recycling policy instituted by China, as well as a similar policy by India (Staub, 2020), that banned the import of most recycled materials and set strict contamination limits on recyclables (Allan, 2018). These nations used to purchase the majority of recycled paper from the United States (Hamel, 2018), which now piles up in landfills. To maintain their engagement and bring home the effects of this policy, the class is asked "*What type of paper products would be rejected under China's National Sword policy?*" After a 5-min discussion, students reply that most paper products contaminated with food, such as pizza boxes, soiled newspaper and paper towels would be rejected. They further conclude that these paper products would end up in a landfill (for slides used during this flipped lesson, see **Supplementary Material 1.2**).

This introduction is followed by a short 5-min lecture on how cardboard and paper products originate from the processing of plants and trees. A figure from Bayer et al. (2007) is used to illustrate the fate of plant material processed by the paper industry, which generates paper products for human consumption. Once used, these products end up in municipal solid waste facilities where some are recycled, composted, or incinerated. The large majority end up in landfills becoming environmental pollution (Bayer et al., 2007).

Before they reflect on the material, students spend a few minutes answering a metacognitive survey question *"Before today, to what extent did you understand the role played by microbes in the biodegradation of paper waste products?"* via clickers (**Supplementary Material 1.2**). Following this, students are asked to predict what happens to paper in a landfill, selecting one of the following options in a clicker question: *"It remains in the landfill, as paper is not degradable," "It will decompose by microbial activity involving respiration," "It will decompose by microbial activity involving fermentation,"* and *"Something else will happen."* Together, these questions provide a baseline

assessment of the students' understanding of the role played by microorganisms in the biodegradation of paper waste products (**Figure 1C** and **Supplementary Figure 2A**).

For the *explore* phase, students spend 10 min researching and reflecting on different aspects of the molecular composition of paper, the microbial communities that degrade paper, the process of exoenzyme secretion, cellulose hydrolysis, and glucose transport and discuss their findings with their table mates. Each student group is divided into 2 subgroups, and each subgroup is tasked with providing the answer to 3 questions (**Supplementary Material 1.2**). One subgroup researches the answers to the following questions: *"What is the molecular composition of cardboard and paper?"*, *"Which microorganisms would degrade cardboard and paper?"*, *"Are these biochemical processes happening aerobically or anaerobically?"*, and *"Which exoenzymes would these organisms use?"*. The second subgroup researches the questions: *"How would these exoenzymes be secreted?"*, *"How would the products of the exoenzymatic reactions be transported into the cytoplasm of the bacteria?"*, and *"Once in the cytoplasm, what biochemical processes would be used in catabolic reactions?"*. For the *explain* phase, the instructors help students synthesize their new knowledge and clarify misconceptions during a 10-min class discussion where students from different tables discuss the answers to these questions as a whole class.

In the second part of the activity, the *elaborate* phase, students spend 15 min applying their knowledge by drawing the entire paper-degradation process in their table groups. The drawing must include the main components of exoenzyme secretion, the enzymatic hydrolysis of cellulose happening outside the cell, the transport of glucose into the cell, and finishing with glycolysis (**Figure 2**). After discussing their drawings, students are again asked to predict what happens to paper in a landfill, and their responses are recorded using clickers (**Figure 1C** and **Supplementary Figure 2B**). Finally, the class spends the last few minutes discussing their predictions and editing their figures to present their final drawing online.

Data Acquisition, Analysis, and Statistics

Students are *evaluated* at multiple points. Formative assessments include metacognitive questions before, during and after class via

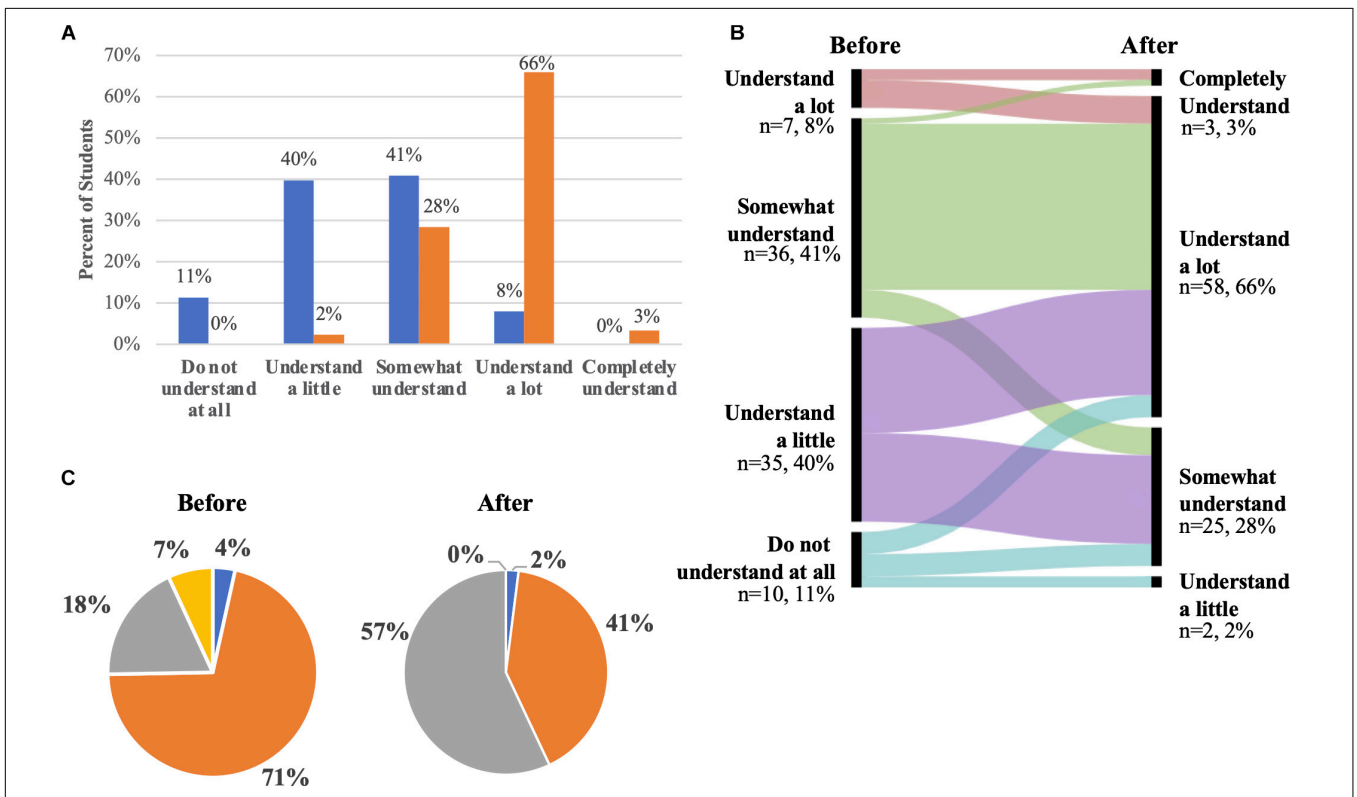

FIGURE 1 | Comparison of student's responses to metacognitive questions before and after the activity. **(A)** Students were asked the question *"To what extent did you understand the role played by microbes in the biodegradation of paper waste products?"* before and after the class activity. The graph displays the responses before the activity (blue bars) and after the activity (orange bars) for the Spring 2020 semester (n = 88). This 5-point Likert survey question was deployed via clickers before the activity or via the Learning Management System after the class. Numbers above the columns represent the percent of students selecting a response. **(B)** Alluvial plot mapping the change in prediction before and after the activity from students in Spring 2020. The left column indicates the predictions before the activity, while the right column indicates the predictions after the activity. Only statements included in the data are shown. **(C)** Pie chart showing student's predictions of the fate of paper before and after the activity. After a brief introduction, students were asked to predict the fate of paper in a landfill via a multiple-choice clicker question. The answer choices included "*It remains in the landfill, as paper is not degradable.*" (blue), "*It will decompose by microbial activity involving respiration.*" (orange), "*It will decompose by microbial activity involving fermentation.*" (gray), and "*Something else will happen.*" (yellow). The graphs display the before (left pie) and after (right pie) responses for the Spring 2020 semesters (n = 88).

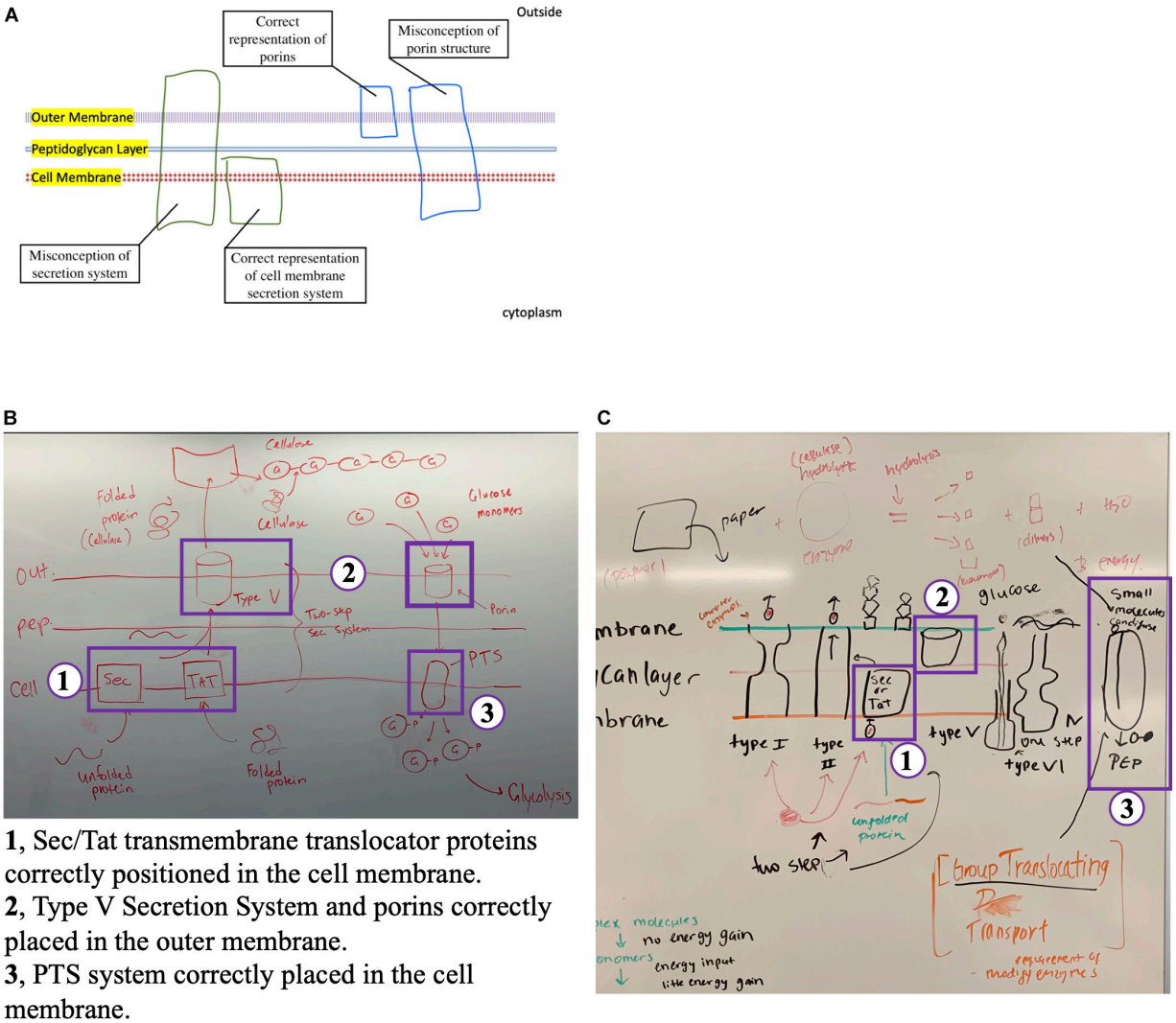

FIGURE 2 | Illustration of enzyme secretion, macromolecule degradation and nutrient transport. Individual groups of students were provided a template to illustrate the processes of enzyme secretion, cellulose hydrolysis and glucose transport. **(A)** Provided template containing the cell envelope of a Gram-negative bacteria, illustrating the correct placement of cell membrane secretion systems and porins, as well as the common misconceptions illustrated by students. **(B)** Correct illustration and **(C)** illustration with misconceptions. The numbers in these images, and the legend, show the most common misconceptions. PTS, phosphotransferase system; Tat, twin-arginine translocation.

clickers or the LMS, drawing their images as well as re-drawing them after in-class discussion. Summative assessments include the readiness assurance quiz and midterm exam questions related to the topic (**Supplementary Materials 2, 3**).

Students in the Spring 2020 semester were given a post-class metacognitive survey via the LMS where they were asked the following metacognitive questions:

(1) What was the most confusing concept in today's class?
(2) Based on today's work, tell us what you think about the following statement: the power of microbes can be harnessed to reach environmentally sustainable goals.
(3) Does today's work illustrate the relationship of microbiology to society? Explain.
(4) Tell us how much you agree with this statement: Today's activity did NOT influence my opinion about recycling paper waste.
(5) Tell us how much you agree with this statement: After today's activity, I will be able to explain how enzyme secretion and sugar transport are related to cell wall and membrane function.

Students in the Fall 2019 cohort only answered the first question. All statistical analyses were conducted in Microsoft Excel version 16.37.

Generation of a codebook

Students responses to the metacognitive question *"What was the most confusing concept in today's class?"* were

analyzed for emergent themes in potential missing knowledge, misinterpretation, and misconceptions of class content, following the process described by Offerdahl and Montplaisir (2014). Briefly, two coders (García-Ojeda and Solis) independently read 20 randomly selected student responses (10 from each cohort) for emerging patterns and established an initial code. This initial code was used to code all 157 submissions (From Fall 2019 and Spring 2020 cohorts). The coders then compared and discussed the initial codes for each independent submission, exploring discrepancies in detail, adding new codes, and collapsing or eliminating other codes. This process was done twice more, using the entire sample, to verify and generate a final codebook (**Supplementary Material 4**). Codes that appeared at least 3 times were kept in the final codebook, consisting of 12 codes in 3 categories. After these discussions, both coders recoded the entire sample. Cohen's kappa values were calculated to determine intercoder reliability (Dewey, 1983; Glen, 2014) using the following equation

$$\kappa = \frac{p_o - p_e}{1 - p_e}$$

where p_o is the relative agreement between both coders, and p_e is the hypothetical probability that agreement was achieved by chance. Kappa values between 0.61–0.8 are considered substantial agreement, while values between 0.81–0.99 are considered near perfect agreement (Landis and Koch, 1977).

Jaccard coefficients have been used to establish the level of consistency between coders (Smith et al., 2013). We calculated the Jaccard coefficient T using the equation $T = n_c/(n_a + n_b - n_c)$, where n_c is the number of times a statement was coded the same by both coders, n_a is the number of times a statement was coded the same by both coders plus the times it was coded by coder 1 but not coder 2, while n_b is the number of times a statement was coded the same by both coders plus the times it was coded by coder 2 but not coder 1. Jaccard coefficients closer to 1 are indicative of high consistency between coders (Niwattanakul et al., 2013).

Word cloud analysis

Word clouds can be used to investigate patters in text data (DePaolo and Wilkinson, 2014). For metacognitive questions 2 and 3 (see "Data Acquisition, Analysis, and Statistics" section for Metacognitive Topic Questions), we identified and ranked key topics emergent in the students' answers by using the online software wordclouds.com.[1] Briefly, we transferred the text responses from the LMS to Microsoft Word and identified and deleted words and phrases that were directly taken from the questions. Such language included phrases like "the power of microbes" or "the relationship of microbiology to society." We also expunged phrases like "I agree because". We then uploaded these .docx files into the wordclouds.com website to create the initial word clouds and produce a term table with the term's respective weights, shown in parenthesis (#, **Supplementary Table 2**).

[1]http://wordclouds.com/

Using the term tables, we narrowed down the number of terms in the word cloud by identifying terms that had similar iterations or meanings and adding their respective weights. For example, the terms Microbes (11), microbes (61) and microbe's (2) were collapsed into the term microbes (74). Words that had meanings unrelated to the question were also eliminated, such as "yes," "yet," "terms," etc. This process reduced the number of terms in the Power of Microbes word cloud from 575 to 126, while reducing the number of terms in the Microbes and Society word cloud from 722 to 248. To reduce the complexity of the word cloud images, we did not include in the illustration terms that appeared less that 4 times.

Adapting to the COVID-19 Pandemic

The COVID-19 pandemic escalated a few weeks into the Spring 2020 semester and university campuses worldwide shut down to prevent the spread of the SARS-CoV2 virus (Richardson, 2020). Since this course is a flipped, hybrid class, transitioning to ERI required minor adjustments. Although the activities described here were deployed in-person before transitioning to ERI, the exam evaluating the content of this activity was administered online. The Spring 2020 students had to navigate the transition period only a week before they took the exam discussed in this paper.

To deter cheating, the exam was open book, open note, timed and delivered via the LMS using Respondus Lockdown Browser® without the Respondus Monitor®. The exam questions were written to build upon foundational knowledge, which require students to use higher order thinking to answer them correctly. Therefore, the answers to these questions could not be easily found online. To clarify exam misconceptions and answer students' questions, the instructors were available via Zoom during the entire exam period.

RESULTS

Formative Assessments

Role of Microorganisms in Paper Degradation

During their pre-class metacognitive survey, we asked students to evaluate their understanding of the role of microbes in paper biodegradation. The majority of students reported improved understanding after the activity (**Figure 1**). Before the activity, over half of students (51%) reported "do not understand at all" or "understand a little," while 41% reported "somewhat understand" and 8% reported "understand a lot" (**Figure 1A**, blue bars). After the activity, the vast majority of students reported, "understand a lot" (66%) or "completely understand" (3%) while only 28% reported "somewhat understand" and few (2%) reported "understand a little" (**Figure 1A**, orange bars).

Using alluvial plots (**Figure 1B**), we examined the changes in reported understanding of the material by comparing their responses at baseline and after the lesson. Most students reported improvement in their understanding of the material. Some students who initially reported "do not understand at all" (**Figure 1B**, light blue) reported "understand a little" after the lesson, while the majority of students in this baseline response

reported either "somewhat understand" or "understand a lot" after the lesson. The group of students who initially answered, "understand a little" (**Figure 1B**, lilac), split equally to reporting "somewhat understand" and "understand a lot" after the lesson. The students initially reporting "somewhat understand" (**Figure 1B**, light green) reported "understand a lot" after the lesson. From students (8%) who reported "understand a lot" at baseline (**Figure 1B**, rose), subsequently reported "completely understand" and "understand a lot" after the lesson. Taken together, our data indicate that students perceived gains in their understanding of the role of microbes in paper biodegradation after completing the lesson.

Mechanism of Paper Biodegradation

Before starting the activity, students were asked to predict the fate of paper in a landfill via a multiple-choice clicker question. Paper buried in a landfill would be degraded primarily by anaerobic mechanisms (Pommier et al., 2010). Initially, the majority of students in the Spring 2020 semester (71%) predicted that respiration played a role in paper biodegradation in landfills (**Figure 1C**). About 18% stated that paper would be degraded via fermentative pathways, and 11% predicted that something else would happen (**Figure 1C**). When asked the same question after the activity, students in the Spring 2020 cohort changed their prediction. Only 41% predicted that paper would be degraded via respiratory mechanisms, while the great majority (57%) stated that fermentation would be the primary pathway of paper degradation (**Figure 1C**). Fewer students (2%) were unsure about the fate of paper in landfills after the activity than before. The greatest transition in answers shifted from students predicting that respiration would play a role to predicting that fermentation would play a role [**Supplementary Figure 2B** (light green)]. Taken together, the majority of students correctly predicted that paper would be degraded anaerobically via fermentative processes after participating in the lesson.

Active Learning Group Exercise: Drawing the Enzyme Secretion, Macromolecule Degradation and Nutrient Transport Pathways

Students demonstrated their overall understanding of class material in a group formative assessment, where they drew the processes of enzyme secretion, cellulose degradation, glucose transport and glucose metabolism (**Figure 2**). This activity was designed to evaluate the extent by which groups of students engaged in model-based reasoning by using a drawing-to-learn approach (Quillin and Thomas, 2015). A group with high mastery of the material would place the Sec/Tat protein secretion systems across the cell membrane, the Type V Secretion System and the porin channels in the outer membrane, and the glucose PTS across the cell membrane (compare **Figures 2B,C**). Their drawings will also show the phosphorylation of glucose to glucose-6-phosphate after transport via the PTS system, leading directly to its hydrolysis via glycolysis. Moreover, they would illustrate cellulose as a polymer as well as have appropriate labels for the cellulase enzyme and other components.

Metacognitive Topic Questions

Student's identified the topics most confusing to them

Question 1: What was the most confusing concept in today's class?

To evaluate this question, we established and validated a 12-item codebook (**Supplementary Material 4**) to explore emerging patterns in students' responses. These codes were subdivided into 3 categories: (1) Concepts, (2) Competencies, and (3) Affect. **Table 2** shows the frequency (f) of these codes for each semester, as well as the Jaccard coefficient (T), and Cohen's kappa ($\kappa \pm$ SE) values evaluating inter-rater reliability. The high Jaccard coefficient ($\geq$0.92) and Cohen's kappa values ($\geq$0.81) indicate a high level of consistency between the two coders.

The Concepts category includes codes that deal with students' questions about class content. In this category, students from both semesters primarily identified Protein Export as the most confusing concept. This code was used 19 times by Fall 2019 students and 27 times by Spring 2020 students. Biochemistry and Misconceptions were more represented in the Fall 2019 cohort compared to the Spring 2020 cohort. To a lesser degree, but with similar frequencies, both cohorts identified Exoenzyme Activity, Location, and Nutrient Transport as confusing topics.

The Competencies category includes codes that describe skills students ought to master over the course of the lesson. Under this category, Big Picture was the most common code (overall frequency of 39 times), found more frequently in the Spring 2020 cohort. Illustration was also frequently cited, (20 times) with the Fall 2019 cohort displaying this code at a higher frequency. Some students in both cohorts reported having challenges with Time to complete the activities (3 times total).

Although not prompted by the question, students from both cohorts expressed ideas about feelings of self-improvement in

TABLE 2 | Codes used to evaluate responses to the question: *"What was the most confusing concept in today's class?"*.

Category[1]	Code	T[2]	κ[3]	SE (κ)	$f_{Fall\,19}$	$f_{Spring\,20}$	f_{Total}[4]
Concepts	Biochemistry	0.99	0.96	0.04	10	4	14
	Exoenzyme activity	0.99	0.89	0.07	5	4	9
	Location	1.00	1.00	0.00	3	2	5
	Misconception	0.99	0.95	0.04	16	6	22
	Nutrient transport	0.97	0.82	0.09	4	6	10
	Protein export	0.98	0.95	0.03	19	27	46
	Other	0.99	0.85	0.15	2	1	3
Competencies	Big picture	0.92	0.81	0.05	14	25	39
	Illustration	0.97	0.89	0.05	12	8	20
	Time	1.00	1.00	0.00	1	2	3
Affect	Concern	0.99	0.89	0.11	2	2	4
	Improvement	0.99	0.96	0.03	12	15	27

[1]Categories: Concepts refer to codes that address students' questions about class content. Competencies refer to codes describing skills students master throughout the course. Affect refers to codes associated with student's feelings. [2]Jaccard coefficient (T) and [3]Cohen's kappa (κ) values were calculated with the combined Fall 2019 and Spring 2020 data (n = 157). [4]Frequency (f) indicates the number of times a code, selected by both coders, appeared in student's responses. Fall 2019 (n = 72), Spring 2020 (n = 85).

conceptual understanding or skills. Some students also expressed feelings of concern about their understanding or feared potential negative consequences for not understanding the material. To reflect the frequency of these statements regarding feelings, we created a category of Affect (**Table 2**). Improvement was the most frequently used code in this category, with both cohorts displaying similar frequencies for this code. Similarly, Concern was also equally represented to a lesser degree in both cohorts.

The power of microbes and the relationship of microbiology to society

> Question 2: Based on today's work, tell us what you think about the following statement: the power of microbes can be harnessed to reach environmentally sustainable goals.

Concerning the power of microbes to reach environmentally sustainable goals, the 10 most used terms (in order of weight) included waste, help, degradation, break, paper, landfill, recycle, clean, decompose and microorganisms (**Figure 3A** and **Supplementary Table 2**). The frequency of use for these terms indicates that students perceived microorganisms as a clean alternative to help the degradation (break or decompose) of waste in landfills. The following student responses were selected because they represent the breadth of perspectives and backgrounds of the students:

> "...Microbes are definitely beneficial for sustainability ... One example can be like bioremediation where microorganisms aid in cleaning air, soil, and water."
>
> "...We could potentially use bacteria to degrade cardboard that does not fall under conditions to be recycled by parties in other countries. We would be able to continue working on sustainability efforts of decreasing soiled cardboard and paper pollution."
>
> "...In the lecture prep videos we were able to see the importance of degradation of oil and petroleum by bacteria using oxygenase for bioremediation."

Students understand the connection to of this microbiology lesson to society

> Question 3: Does today's work illustrate the relationship of microbiology to society?

Concerning the relationship of microbiology to society, students connected the activity to four overall societal applications, (1) waste management, (2) sustainability and environmental solutions to pollution, (3) biodegradation as a tool, and (4) energy cycles essential for human life (**Figure 3B** and **Supplementary Table 2**). In particular, students reported that the activity helped them understand the real-world implications of the biology they were learning:

> "... Listening to the newscast about the garbage problem with China really made it apparent to me how important it is for us in America to find a reliable, safe, and sustainable solution to the ever-increasing garbage problem. I see how microbes can be used to help break down certain types of waste and I am very curious to see if there will be newer research and findings regarding microbe usage in the waste management sector."
>
> "The activity helped me to connect everything together especially by relating it to the current problem we have with paper not being properly recycled. The activity helped me envision how paper was broken down via cellulase enzyme then brought in by porins and PTS systems and through catabolic reactions we harvested energy. The activity made everything full circle for me."

Students agree: the lesson influences their opinion about paper recycling

> Question 4: Tell us how much you agree with this statement: Today's activity did NOT influence my opinion about recycling paper waste.

To evaluate if the lesson influenced the students' opinion about paper recycling, we deployed the above 5-point Likert scale question via LMS. Most students either Strongly disagreed (33%) or Somewhat disagreed (36%) that the activity did not influenced their opinion about recycling paper waste (**Figure 4A**). About a fifth of the students (22%) Neither agreed nor disagreed with the statement, while less than 10% of the students Somewhat agreed or Strongly agreed.

Students agree: the lesson increases their confidence to explain the relationship between enzyme secretion, and sugar transport to cell wall and membrane structure

> Question 5: Tell us how much you agree with this statement: After today's activity, I will be able to explain how enzyme secretion and sugar transport are related to cell wall and membrane function.

We evaluated the students' confidence in their ability to explain the relationship between enzyme secretion and nutrient transport to cell wall and membrane structure. After the lesson, we asked students to rate how much they disagreed or agreed with the question above. Most students (81.8%) reported that they could explain how enzyme secretion and sugar transport are related to cell wall and membrane function. About 9% of the students were neutral about this statement, while very few of them either "strongly disagreed" (4.5%) or "somewhat disagreed" (4.5%) with the statement (**Figure 4B**).

Summative Assessments

Readiness Assurance Quiz

During the first 10 min of class, students took a 5-point quiz containing 5 multiple choice questions to test their comprehension of the video lectures (**Supplementary Material 1.1**). Both cohorts of students performed similarly on the quiz ($t[176] = 0.44$, $p = 0.66$). The Fall 2019 class scored 3.6 ± 1.1 points (mean $\pm$ SD, 71%) and the Spring 2020 class scored 3.5 ± 0.9 points (70%). Students were successful at understanding how exoenzymes hydrolyze polymers (questions 3–5) but were less successful in describing where in the cell these processes are happening and how the products of hydrolysis are transported (questions 1–2).

Midterm Exam

The midterm questions were designed to assess the students' mastery of the lesson's core concepts (Crowe et al., 2008) and have evolved over the development of the course. All exams

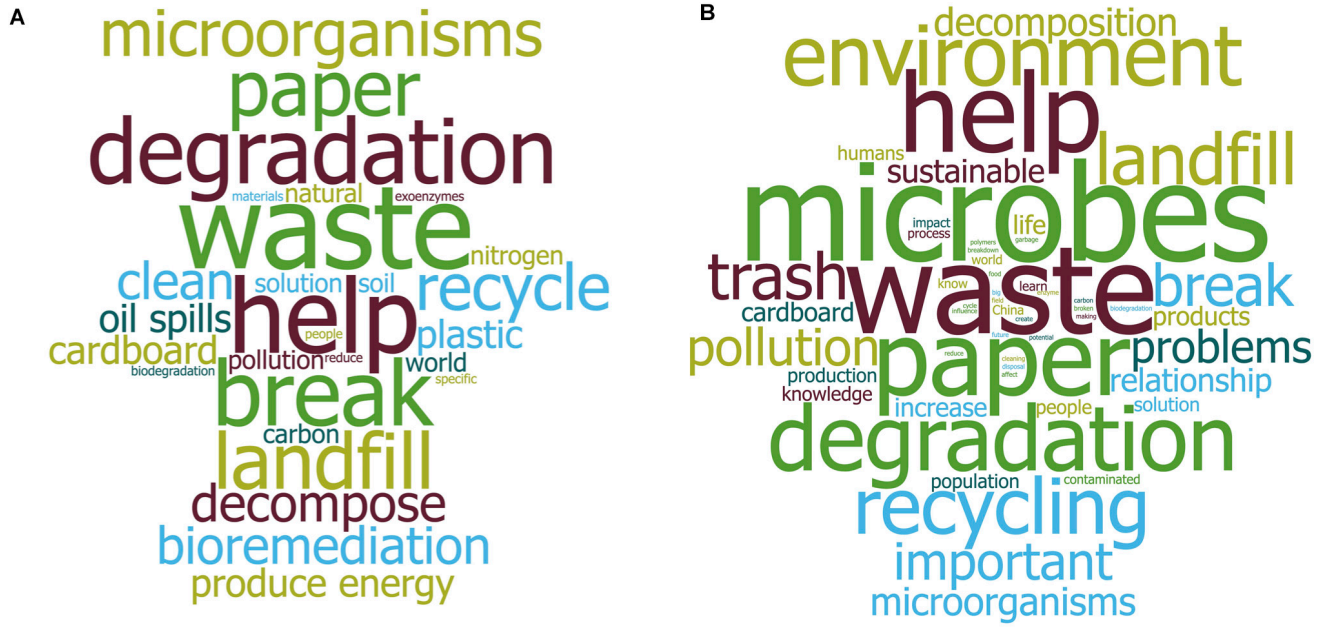

FIGURE 3 | Word clouds illustrating terms in the responses to metacognitive questions. **(A)** Microbes and environmental goals. Following the activity, students were asked the question: *Based on today's work, tell us what you think about the following statement: the power of microbes can be harnessed to reach environmentally sustainable goals.* **(B)** The relationship between microbiology and society. Following the activity, students answer the question: *Does today's work illustrates the relationship of microbiology to society? Explain.* Both of these free-response survey questions were deployed via the Learning Management System after the class. Text from the student's responses was organized using a word cloud software that highlights, by word size, how often a term was used. The images display the responses from the Spring 2020 semester (n = 88). To simplify the image, only terms used at least 4 times are shown.

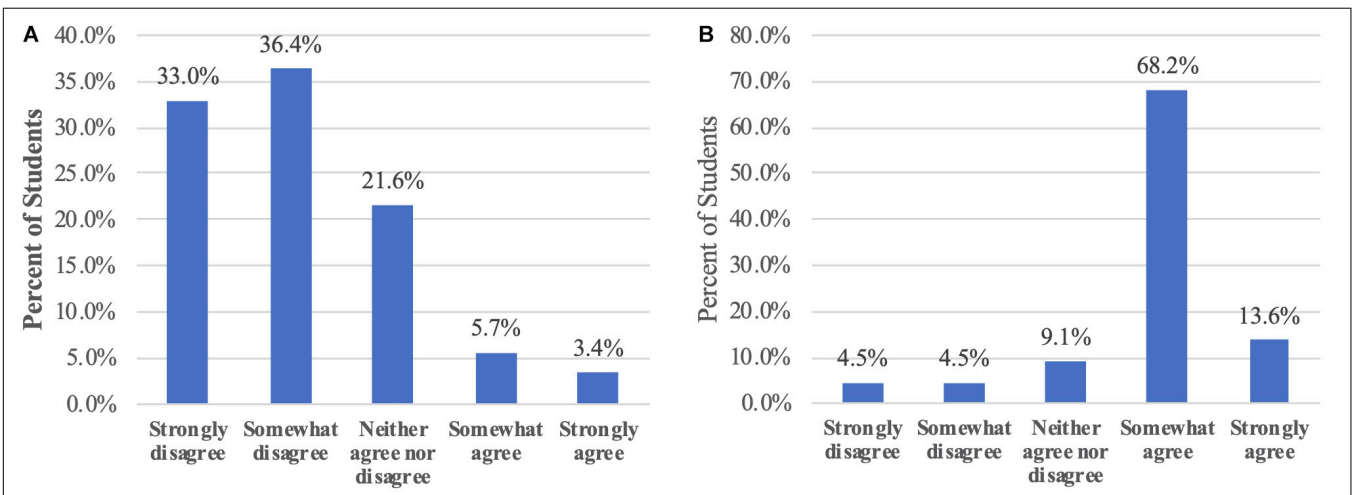

FIGURE 4 | (A) The activity strongly influences student's opinions about recycling. Following the activity, students were asked: "*Tell us how much you agree with this statement: Today's activity did NOT influence my opinion about recycling paper waste.*" **(B)** The activity strongly enhances student's understanding of enzyme secretion and nutrient transport. Following the activity, students were asked: "*Tell us how much you agree with this statement: After today's activity, I will be able to explain how enzyme secretion and sugar transport are related to cell wall and membrane function.*" These 5-point Likert survey questions were deployed via the Learning Management System after the class. The graphs display the responses from the Spring 2020 semester (n = 88). Numbers above the columns represent the percent of students selecting a response.

for this course are open-ended with brief essay responses. For context, after making the course hybrid in Fall 2019, the exams shifted from asking low-order questions to asking students higher-order synthesis questions about the entire process of macromolecule degradation (**Supplementary Material 6**). In Fall 2018, students averaged 71% on the questions relevant to this lesson, providing basic answers about exoenzyme identification and secretion systems (data not shown). However, students

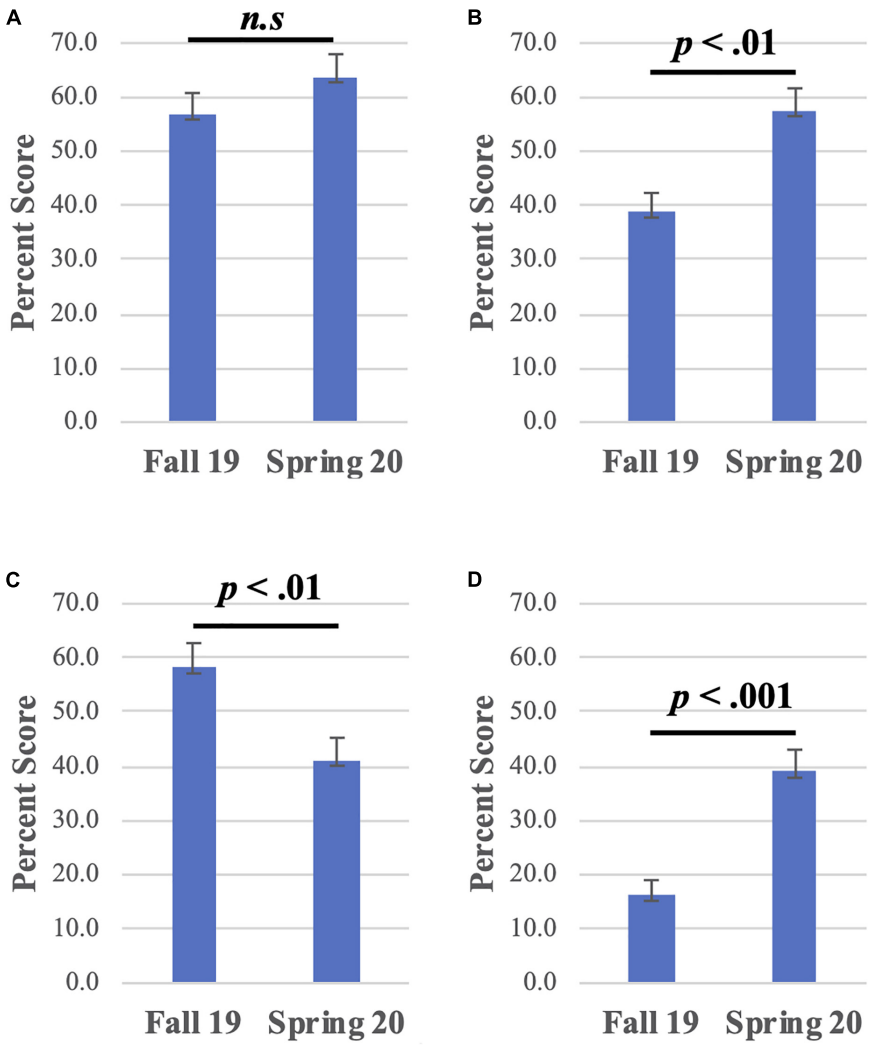

FIGURE 5 | Comparison of exam performance in summative assessment questions for the Fall 2019 and the Spring 2020 cohorts. All graphs compare the mean percentage score and error bars are the standard error of the mean. **(A)** Question 5a: Protein Secretion, $t(177) = -1.20$, $p = 0.232$. **(B)** Question 5b: Exoenzyme Function, $t(177) = -3.24$, $p = 0.001$. **(C)** Question 5c: Nutrient Transport, $t(177) = 2.63$, $p = 0.009$. **(D)** Question 5d, Metabolism, $t(177) = -4.68$, $p < 0.00001$. Bar graphs display the responses for Fall 2019 ($n = 89$) and Spring 2020 semester ($n = 90$). All statistics are unpaired, two-tailed, Student's *t*-Tests. For details on the questions and their answers, please see the **Supplementary Material**.

did not demonstrate mastery of the entire macromolecule degradation process. In Spring 2019, students were challenged to describe the degradation process of a protein without the problem-based learning exercise. Their answers averaged 31% for these questions (data not shown). Overall, students from the pre-hybrid course demonstrated a rudimentary understanding of the processes of macromolecule degradation but were challenged when connecting macromolecule degradation to a non-carbohydrate substrate. This demonstrates a breakdown of knowledge with the macromolecule degradation process, thereby inspiring the shift of the questions to the higher-level processes assessed here.

In the exam questions pertaining to this lesson, students are asked to detail the process of decomposition and metabolism of a triglyceride to their final carbon-based products (CO_2 or a fermentation product). This shift in substrate in the exam, from cellulose used in the lesson, would allow us to ascertain if students transferred the knowledge obtained in the lesson to a new scenario (Nokes-Malach and Mestre, 2013). The exam question had four parts assessing students' mastery of protein secretion, exoenzyme function, nutrient transport and metabolism. For both semesters, these exam questions were graded by the same grader, using the same key, and the same rubric (**Supplementary Material 3**). These two cohorts were the first to be assessed with this question.

When comparing the entirety of the exam, both cohorts performed similarly ($t[176] = 1.08$, $p = 0.28$). The Fall 2019 cohort had a mean score of 77% and the Spring 2020 had a mean score of 74%. Since Fall 2019, students do not keep their exams after reviewing the material in discussion so there is little chance that

the Spring 2020 cohort had access to exam questions ahead of time. Both cohorts demonstrated similar comprehension of the exoenzyme secretion process (**Figure 5A**). Students in the Spring semester cohort performed better than the Fall semester cohort in the question concerning Exoenzyme function (**Figure 5B**). On the other hand, Fall semester students performed better in the question relating to nutrient transport (**Figure 5C**). Although the Spring 2020 students performed better, both cohorts had challenges identifying the products of triglyceride degradation (glycerol and fatty acids) and placing these within the beta oxidation pathway to generate Acetyl-CoA (**Figure 5D**).

DISCUSSION

Practical Implications and Lessons Learned

Peer-led, team-based learning is known to give students an opportunity to develop positive interdependence, scientific reasoning, critical thinking, and communication skills (Scager et al., 2020; Trempy et al., 2002). Additionally, peer-led learning provides a more inclusive and supportive learning environment, particularly to underrepresented students who come from culturally interdependent communities (Covarrubias et al., 2016). General Microbiology utilizes project-based learning in the TEAL classroom environment to facilitate the exploration of real-world scenarios (Dourmashkin et al., 2020) and help students synthesize their own understanding of material (Leupen, 2020). This lesson helped students connect first-hand with the global impact of paper pollution and the role microbes play in the biodegradation of paper waste. Students demonstrated a deep understanding and increased awareness of the societal issues surrounding paper waste management and sustainability.

Illustrating the entire biodegradation process of cellulose was the most challenging part of the activity, as students are required to engage in systems thinking, bringing together the material not only from this lesson, but from two other previous lessons (cell wall structure and nutrient transport). This activity revealed misconceptions in students' understanding of the material (**Figures 2A,C**). Some students found it difficult to differentiate between the Sec and Tat protein translocation system, centered around a knowledge breakdown between which system translocated unfolded (Sec) versus folded (Tat) proteins. Furthermore, students were confused by which protein secretion system was used to transport proteins across the outer membrane (Type V Secretion System) and where these secretion systems were located in the cell envelope. This in-class evidence was confirmed through the coding of the post-class metacognitive response to "*What is the most confusing concept in today's class?*", where Protein Export was the most commonly represented code, followed by Big Picture and Illustration (**Table 2**). Another misconception centered on the transport of cellulose, as some students misidentified cellulose as a monomer and missed including glucose in their diagrams. Concerning nutrient metabolism, some students had challenges ascertaining the biochemical pathways utilized to digest glucose monomers as a source of energy. These topics also surfaced in the coding of the answers to the metacognitive question "*What is the most confusing concept in today's class?*", where Biochemistry, Nutrient Transport and Exoenzyme Activity codes were abundantly represented (**Table 2**). Combined, these misconceptions demonstrated a general challenge with visualizing structure/function relationships in the cell envelope. This informed us of the importance of emphasizing the structural components of the lesson for future students. In the next iterations of the class, we will prime students to review these topics during the preparatory stage of the carbon cycle lesson. In this way, the aspects that are least understood to the class would be emphasized during individual lectures and incorporated into activities to solidify this knowledge.

Overall, students were effective at explaining how exoenzymes are secreted (**Figure 5A**), their role in macromolecule hydrolysis (**Figure 5B**) as well as the transport of their fatty acid products into the cell (**Figure 5C**) but were less effective at connecting the biochemical pathway for fatty acid metabolism to energy production. We strategically chose to ask students about triglyceride hydrolysis instead of cellulose to provide them the opportunity to demonstrate their ability to transfer the knowledge they gained from the cellulose lesson to the triglyceride exam question (Nokes-Malach and Mestre, 2013). In this regard, students showed a breakdown of knowledge in the level of completion in their answer regarding the specific pathway by which fatty acids are processed (**Figure 5D**). For example, some students would correctly state that fatty acids are processed into acetyl-CoA via beta oxidation but could not follow through connecting acetyl-CoA to the Krebs cycle and the electron transport chain. Some students mentioned the Krebs cycle and electron transport chain, but not the beta oxidation pathway. This breakdown may reflect the fact that these questions were the most challenging on the exam and required students to think at a higher-cognitive level than other questions. Moreover, students did not have the opportunity to practice transferring knowledge of the various macromolecule degradation processes from one substrate to another before the exam. This signaled to the instructors to consider providing more opportunities to practice this skill, particularly in the asynchronous and homework. While the exam scores were low, they were an improvement from the scores of the pre-hybrid Spring 2019 cohort, which did not have the aid of the problem-based learning exercise (data not shown). This demonstrates an improvement between the pre-hybrid and hybrid cohorts when approaching higher-cognitive level questions.

Based on their formative assessment results, students showcased evidence of their learning and achievement of the lesson's learning outcomes. This is illustrated in the transition in predictions about the fate of paper in a landfill (**Figure 1C** and **Supplementary Figure 2**). Most students came to class incorrectly believing that respiration played a role in the biodegradation of paper waste products. By the end of the activity, most students stated that fermentation plays a primary role in this process. However, a good proportion of students (41%, **Figure 1C**) still stated that paper would be degraded via respiratory processes. We hypothesize that the lack of clear understanding of landfill architecture as well as images

used during the introduction to the activity of paper exposed to air in landfills might have influenced student's answers (**Supplementary Material 1.2**). In future iterations of the class, we will ensure to reinforce these concepts in the pre-flipped lecture preparation material.

Students were able to visually work through the mechanism of nutrient transport and correctly illustrate the biochemical processes accurately. Misconceptions of the structural organization of these processes were identified quickly and resolved through visual exploration and discussion (Schnotz, 2014). Our metacognition survey demonstrated that students self-identified their growth in understanding before and after the activity (**Figure 4B**). The students synthesized and demonstrated their understanding through the summative assessments, being able to recall the process of protein secretion and nutrient absorption but struggled to transfer knowledge from one polymer (cellulose) to another (triglycerides). Future class iterations will provide discussion forum questions and homework that will give students more opportunities to examine a variety of macromolecules and work through the entire biochemical pathways needed for their degradation.

Conclusions and Recommendations

Using a variety of teaching strategies and technology, this lesson departed from a traditional teaching model by giving students an opportunity to address a real-world problem. Students demonstrated their learning by building their own systems-level understanding of the microbiological and biochemical processes involved in the breakdown of paper. This lesson was facilitated by, and took advantage of, the TEAL lab learning environment and a hybrid online model, both components that may not be accessible to all universities. The TEAL lab facilitates the use of active learning, but it is neither required to use these strategies nor required to create a collaborate learning environment. There are many ways to foster collaborative learning including structured discussion, reciprocal teaching, and problem-solving (Barkley et al., 2014) that are independent of the TEAL lab. For courses that do not have access to online resources, face-to-face interactions can produce statistically similar grades from online learning as well as increased levels of student satisfaction (Summers et al., 2005).

There are some inherent challenges that come with implementing a flipped lesson with evidence-based teaching strategies of this nature. It takes a significant amount of time to prepare each component, which requires intention and alignment with the learning outcomes to be effective for student learning and engagement. Additionally, these active learning strategies may be new to instructors accustomed to traditional teaching and may require additional training to model effectively. To facilitate the amount of activity in the given time, the timing during flipped lecture needs to be managed closely to incorporate all the components of a 5E model lesson. Timing itself could be a limitation if courses are less than the 75 min discussed here and if instructors feel there is not enough time to cover the content with active learning (Graffam, 2007). This lesson may be difficult to implement in a large (>200 person) class and may require additional levels of organization or facilitation. Furthermore, there may be systemic resistance to incorporating active learning techniques (Bathgate et al., 2019) that faculty may need to overcome without reward (Michael, 2007).

In order to increase content retention and reduce misconceptions, students could perform a pre-lecture homework where they can review the materials discussed in the lesson's video lectures as well as from previous coursework. We found that, overall, students improved their understanding of the concepts after the 5E lesson. Where some students struggled was drawing the structures related to the biological processes. We recommend that instructors incorporate more model drawing activities to help identify misconceptions and provide students with an opportunity to promote reasoning skills (Quillin and Thomas, 2015). We also recommend offering a variety of examples for students to practice transferring their knowledge from one example to another. Lastly, we recommend and encourage faculty to center activities, when possible, on real-world scenarios that are relevant to students, especially scenarios that impact underserved student communities, to connect the concepts and their implications to student experiences (Harackiewicz et al., 2016).

SUMMARY

Today's students suffer from the burden of climate change and global pollution and need to develop skills to think critically about these problems. Problem-based learning can engage students in real-world scenarios while simultaneously learning complex microbiological and biochemical concepts. By using deliberate teaching strategies, outlined in this lesson, we demonstrate increased student conceptual understanding and perceived understanding of microbial carbon assimilation and its role in paper waste degradation. We coded student responses from a reflective survey and identified common misconceptions and perceived gains. Student performance was measured through a variety of assessments including a drawing activity and exam questions. We were able to determine areas where student performance could improve and address them accordingly. We recommend instructors consider using real-world scenarios when teaching complex topics to foster student engagement and interest in the topic in and beyond the classroom.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by UC Merced Institutional Review Board.

The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

MG-O conceived the lesson's idea with assistance from JS. MG-O and JS developed the learning outcomes, hybrid flipped classroom format, and created course materials such as the video lectures and assessments. MG-O and RS developed the codebook and coded the metacognitive responses. MG-O created and edited figures and tables as well as performed the statistical analyses. JS wrote the manuscript with contributions from MG-O and RS with editing and revisions from MG-O. All authors discussed the results and contributed to development of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.588918/full#supplementary-material

REFERENCES

AAAS (2009). "Vision and change in undergraduate biology education: a call to action," in *Proceedings of the American Association for the Advancement of Science National Conference Report*, Washington, DC.

Allan, R. (2018). China puts recycled to the sword. *Appita* 71:202.

Allen, D., and Tanner, K. (2005). Infusing active learning into the large-enrollment biology class: seven strategies, from the simple to complex. *Cell Biol. Educ.* 4, 262–268. doi: 10.1187/cbe.05-08-0113

Allen, D. E., Donham, R. S., and Bernhardt, S. A. (2011). Problem-based learning. *New Direct. Teach. Learn.* 2011, 21–29. doi: 10.1002/tl.465

Arik, S., and Yilmaz, M. (2020). The effect of constructivist learning approach and active learning on environmental education: a meta-analysis study. *Int. Electron. J. Environ. Educ.* 10, 44–84.

Barkley, E. F., Cross, K. P., and Major, C. H. (2014). *Collaborative Learning Techniques: A Handbook for College Faculty*. Hoboken, NJ: John Wiley & Sons.

Bathgate, M. E., Aragón, O. R., Cavanagh, A. J., Frederick, J., and Graham, M. J. (2019). Supports: a key factor in faculty implementation of evidence-based teaching. *CBE Life Sci. Educ.* 18:ar22. doi: 10.1187/cbe.17-12-0272

Bayer, E. A., Lamed, R., and Himmel, M. E. (2007). The potential of cellulases and cellulosomes for cellulosic waste management. *Curr. Opin. Biotechnol.* 18, 237–245. doi: 10.1016/j.copbio.2007.04.004

Beichner, R. J., Saul, J. M., Abbott, D. S., Morse, J. J., Allain, R. J., Bonham, S. W., et al. (2007). The student-centered activities for large enrollment undergraduate programs (SCALE-UP) project. *Res. Based Reform Univ. Phys.* 1, 2–39.

Brand, M. (2018). *How Much of Our Stuff Actually Gets Recycled? | Press Play*. London: KCRW.

Bybee, R. W. (1997). *Achieving Scientific Literacy: From Purposes to Practices*. Portsmouth: Heinemann.

Cakır, N. K. (2017). Effect of 5E learning model on academic achievement, attitude and science process skills: meta-analysis Study. *J. Educ. Train. Stud.* 5:157. doi: 10.11114/jets.v5i11.2649

Chorazy, M. L., and Klinedinst, K. S. (2019). Learn by doing: a model for incorporating high-impact experiential learning into an undergraduate public health curriculum. *Front. Public Health* 7:31. doi: 10.3389/fpubh.2019.00031

Cotner, S., Loper, J., Walker, J. D., and Brooks, D. C. (2013). "It's Not You, It's the Room"—are the high-tech, active learning classrooms worth it? *J. College Sci. Teach.* 42, 82–88. doi: 10.2505/4/jcst13_042_06_82

Covarrubias, R., Herrmann, S. D., and Fryberg, S. A. (2016). Affirming the interdependent self: implications for latino student performance. *Basic Appl. Soc. Psychol.* 38, 47–57. doi: 10.1080/01973533.2015.1129609

Crowe, A., Dirks, C., and Wenderoth, M. P. (2008). Biology in bloom: implementing bloom's taxonomy to enhance student learning in biology. *CBE Life Sci. Educ.* 7, 368–381. doi: 10.1187/cbe.08-05-0024

DePaolo, C. A., and Wilkinson, K. (2014). Get your head into the clouds: using word clouds for analyzing qualitative assessment data. *TechTrends* 58, 38–44. doi: 10.1007/s11528-014-0750-9

Desa, A., Kadir, N. B. A., and Yusooff, F. (2011). A study on the knowledge, attitudes, awareness status and behaviour concerning solid waste management. *Proc. Soc. Behav. Sci.* 18, 643–648. doi: 10.1016/j.sbspro.2011.05.095

Desilver, D. (2016). *Recycling Perceptions, Realities Vary Widely from Place to Place [Fact Tank]*. Washington, DC: Pew Research Center.

Dewey, M. E. (1983). Coefficients of agreement. *Br. J. Psychiatry* 143, 487–489. doi: 10.1192/bjp.143.5.487

Dourmashkin, P., Tomasik, M., and Rayyan, S. (2020). "The TEAL physics project at MIT," in *Active Learning in College Science: The Case for Evidence-Based Practice*, eds J. J. Mintzes and E. M. Walter (Berlin: Springer International Publishing), 499–520. doi: 10.1007/978-3-030-33600-4_31

Freeman, S., Eddy, S. L., McDonough, M., Smith, M. K., Okoroafor, N., Jordt, H., et al. (2014). Active learning increases student performance in science, engineering, and mathematics. *Proc. Natl. Acad. Sci. U.S.A.* 111, 8410–8415. doi: 10.1073/pnas.1319030111

Gilboy, M. B., Heinerichs, S., and Pazzaglia, G. (2015). Enhancing student engagement using the flipped classroom. *J. Nutr. Educ. Behav.* 47, 109–114. doi: 10.1016/j.jneb.2014.08.008

Glen, S. (2014). *Cohen's Kappa Statistic. Statistics How To: Elementary Statistics for the Rest of Us!*. Available online at: https://www.statisticshowto.com/cohens-kappa-statistic/ (accessed July 20, 2020).

Graffam, B. (2007). Active learning in medical education: strategies for beginning implementation. *Med. Teach.* 29, 38–42. doi: 10.1080/01421590601176398

Hamel, J. (2018). *The China Ban: Why More of Your Recyclables are Going to the Landfill*. London: KCRW.

Harackiewicz, J. M., Smith, J. L., and Priniski, S. J. (2016). Interest matters: the importance of promoting interest in education. *Policy Insights Behav. Brain Sci.* 3, 220–227. doi: 10.1177/2372732216655542

Landis, J. R., and Koch, G. G. (1977). The measurement of observer agreement for categorical data. *Biometrics* 33, 159–174. doi: 10.2307/2529310

Leupen, S. (2020). "Team-based learning in STEM and the health sciences," in *Active Learning in College Science: The Case for Evidence-Based Practice*, eds J. J. Mintzes and E. M. Walter (Cham: Springer International Publishing), 219–232. doi: 10.1007/978-3-030-33600-4_15

Marope, P. T. M. (2016). Brain science, education, and learning: making connections. *Prospects* 46, 187–190. doi: 10.1007/s11125-017-9400-2

Michael, J. (2007). Faculty perceptions about barriers to active learning. *College Teach.* 55, 42–47. doi: 10.3200/CTCH.55.2.42-47

Michaelsen, L. K., and Sweet, M. (2011). Team-based learning. *New Direct. Teach. Learn.* 2011, 41–51. doi: 10.1002/tl.467

Nelson-Hurwitz, D. C., and Buchthal, O. V. (2019). Using deliberative pedagogy as a tool for critical thinking and career preparation among undergraduate public health students. *Front. Public Health* 7:37. doi: 10.3389/fpubh.2019.00037

Niwattanakul, S., Singthongchai, J., Naenudorn, E., and Wanapu, S. (2013). Using of jaccard coefficient for keywords similarity. *Proc. Int. Multiconf. Eng. Comput. Sci.* 1, 380–384. doi: 10.12720/lnit.1.4.159-164

Nokes-Malach, T. J., and Mestre, J. P. (2013). Toward a model of transfer as sense-making. *Educ. Psychol.* 48, 184–207. doi: 10.1080/00461520.2013.807556

Offerdahl, E. G., and Montplaisir, L. (2014). Student-generated reading questions: diagnosing student thinking with diverse formative assessments. *Biochem. Mol. Biol. Educ.* 42, 29–38. doi: 10.1002/bmb.20757

Orsmond, P., and Zvauya, R. (2015). Community of learners: charting learning in first year graduate entry medical students during problem-based learning (PBL) study. *Adv. Health Sci. Educ.* 20, 479–497. doi: 10.1007/s10459-014-9542-4

Pommier, S., Llamas, A. M., and Lefebvre, X. (2010). Analysis of the outcome of shredding pretreatment on the anaerobic biodegradability of paper and cardboard materials. *Bioresour. Technol.* 101, 463–468. doi: 10.1016/j.biortech.2009.07.034

Quillin, K., and Thomas, S. (2015). Drawing-to-learn: a framework for using drawings to promote model-based reasoning in biology. *CBE Life Sci. Educ.* 14:es2. doi: 10.1187/cbe.14-08-0128

Richardson, S. (2020). *Research News Roundup: How universities are responding to the COVID-19 pandemic [Research News]*. Jerusalem: Ex Libris.

Scager, K., Boonstra, J., Peeters, T., Vulperhorst, J., and Wiegant, F. (2020). "Collaborative learning in college science: evoking positive interdependence," in *Active Learning in College Science: The Case for Evidence-Based Practice*, eds J. J. Mintzes and E. M. Walter (Cham: Springer International Publishing), 233–247. doi: 10.1007/978-3-030-33600-4_16

Schnotz, W. (2014). "Integrated model of text and picture comprehension," in *The Cambridge Handbook of Multimedia Learning*, 2nd Edn, ed. R. E. Mayer (Cambridge, MA: Cambridge University Press), 72–103. doi: 10.1017/CBO9781139547369.006

Smith, M. K., Jones, F. H. M., Gilbert, S. L., and Wieman, C. E. (2013). The classroom observation protocol for undergraduate stem (COPUS): a new instrument to characterize university STEM classroom practices. *CBE Life Sci. Educ.* 12, 618–627. doi: 10.1187/cbe.13-08-0154

Snyder, J. J., Sloane, J. D., Dunk, R. D. P., and Wiles, J. R. (2016). Peer-led team learning helps minority students succeed. *PLoS Biol.* 14:e1002398. doi: 10.1371/journal.pbio.1002398

Staub, C. (2020). *Details on India's Mixed Paper Import Crackdown*. New York, NY: Resource Recycling News.

Summers, J. J., Waigandt, A., and Whittaker, T. A. (2005). A comparison of student achievement and satisfaction in an online versus a traditional face-to-face statistics class. *Innov. High. Educ.* 29, 233–250. doi: 10.1007/s10755-005-1938-x

Trempy, J. E., Skinner, M. M., and Siebold, W. A. (2002). Learning microbiology through cooperation: designing cooperative learning activities that promote interdependence, interaction, and accountability. *Microbiol. Educ.* 3, 26–36. doi: 10.1128/154288102X14285807693547

von Glasersfeld, E. (1987). *The Construction of Knowledge, Contributions to Conceptual Semantics*. London: Intersystems Publications.

Wiggins, G., and McTighe, J. (1998). What is backward design? *Understand. Design* 1, 7–19.

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Combining Microbial Culturing With Mathematical Modeling in an Introductory Course-Based Undergraduate Research Experience

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Quantitative techniques are a critical part of contemporary biology research, but students interested in biology enter college with widely varying quantitative skills and attitudes toward mathematics. Course-based undergraduate research experiences (CUREs) may be an early way to build student competency and positive attitudes. Here we describe the design, implementation, and assessment of an introductory quantitative CURE focused on halophilic microbes. In this CURE, students culture and isolate halophilic microbes from environmental and food samples, perform growth assays, then use mathematical modeling to quantify the growth rate of strains in different salinities. To assess how the course may impact students' future academic plans and attitudes toward the use of math in biology, we used pre- and post-quarter surveys. Students who completed the course showed more positive attitudes toward science learning and an increased interest in pursuing additional quantitative biology experiences. We argue that the classroom application of microbiology methods, combined with mathematical modeling using student-generated data, provides a degree of student ownership, collaboration, iteration, and discovery that makes quantitative learning both relevant and exciting to students.

Keywords: CURE, microbial diversity, microbiome, microbial culturing, mathematical modeling, education, logistic growth curve analysis

INTRODUCTION

The American Association for the Advancement of Science and the National Research Council have each called for renewed undergraduate education efforts to build broadly applicable biology research skills (National Research Council, 2003, 2009; American Association for the Advancement of Science, 2011). One report, *Vision and Change* (American Association for the Advancement of Science, 2011), laid out influential and ambitious goals for reforming undergraduate biology

education, encouraging the integration of core concepts and competencies throughout the curriculum. Several of these competencies are quantitative, including the ability to use quantitative reasoning and the ability to apply modeling and simulation.

However, quantitative material can be challenging to introduce to biology-interested students early in their undergraduate career. Students enter college with a wide range of mathematics skills (Treisman, 1992; Agustin and Agustin, 2009; Sonnert and Sadler, 2014), and experiences in traditional introductory courses like calculus might lead some students to leave STEM (Ellis et al., 2016). In addition, many undergraduate biology students may also have unfavorable emotions about math (Wachsmuth et al., 2017). These emotions can translate to poor performance in math-related coursework (Ma and Kishor, 1997).

One way to address these challenges is by integrating math and biology coursework at multiple points along an undergraduate curriculum (Bialek and Botstein, 2004; Chiel et al., 2010; Depelteau et al., 2010; Duffus and Olifer, 2010; Miller and Walston, 2010; Aikens and Dolan, 2014; Eaton and Highlander, 2017). In our experience, however, few introductory biology lab courses emphasize the breadth of quantitative skills commonly used in biology research. We propose that introductory course-based undergraduate research experiences (CUREs) may be a valuable early part of this type of integrated curriculum, given their potential positive effects on student learning and attitudes.

CUREs are natural candidates to promote quantitative learning and build positive attitudes toward math among biology-interested students. These courses engage students in the practice of research from within the classroom, emphasizing peer collaboration and iterative approaches to the research process while students use modern scientific practices to address novel, broadly relevant research questions (Auchincloss et al., 2014). Student participation in CUREs can benefit student learning as well as persistence in STEM and attitudes toward science (Brownell et al., 2012; Jordan et al., 2014; Olimpo et al., 2016; Rodenbusch et al., 2016; reviewed by Dolan, 2016), and these courses may provide an avenue toward creating a more inclusive academic environment (Bangera and Brownell, 2014). Several recent CUREs have included quantitative learning outcomes and found student benefits (Brownell et al., 2015; Kirkpatrick et al., 2019; Murren et al., 2019), although these courses typically focus more on data and figure interpretation than on mathematical modeling.

In this study we outline an introductory quantitative biology CURE that combines microbial culturing and genomic DNA isolation with modeling and quantitative characterizations of growth rate. We assessed student attitudinal gains using several published instruments (Andrews et al., 2017; Lopatto et al., 2008; Shaffer et al., 2010), as well as short-answer questions related to students' future course and career plans. We sought to answer three questions.

1. Would this quantitative biology CURE increase students' interest in and perceived utility of using math in biology?
2. Would this course help students develop more positive attitudes toward science learning?
3. Does this course influence student plans for future quantitative courses or careers?

We hypothesized that this course might increase students' desire to pursue future quantitative biology experiences by building more positive attitudes toward science learning and toward using math in biology. Assessing changes in student attitudes toward math in biology proved difficult due to strongly positive initial attitudes in this self-selected population. However, we find some evidence of positive changes in student attitudes toward learning science, as well as increased student interest in pursuing future quantitative experiences.

MATERIALS AND METHODS

Developing the Course Structure and Subject

Here we outline the process we followed in creating a quantitative biology CURE. We developed the course to help students build quantitative skills that are commonly used in biology research. To that end, we informally surveyed the laboratory and quantitative skills used in local microbiology research labs. We identified commonly used lab skills including microbial culturing, microscopy, and spectrophotometry, which integrated with quantitative skills like calculations of concentration and dilution factors as well as mathematical modeling of growth curves. We chose to modify an existing workflow that is commonly used in undergraduate research on the microbiome (Dunitz et al., 2015). In this workflow, students culture microbes from almost any environmental sample, generate isolates from the sample(s), and use growth curves of the isolates to quantify aspects of the organism's biology. The workflow was initially piloted as a less quantitatively oriented seminar course, using environmental samples ranging from nectar (Dahlhausen, 2018) to abalone (Vater et al., 2016) to koala feces (Coil, 2017). These courses and other CUREs have been discussed by Vater et al. (2019). In our more quantitatively focused version of this microbial isolation approach, the specific taxa play a minimal role in shaping the course learning goals, teaching methods, and assessments.

The current iteration of this CURE focuses on culturing, isolating, and quantitatively characterizing the features of halophiles (Rodriguez-Medina et al., 2020). Halophiles are a category of microorganisms that thrive in hypersaline conditions from sea salinity to saturation. These organisms span all three domains of life and can be found in diverse global environments including hypersaline soils, lakes, solar salterns, deep salt mines, and natural brines in coastal and submarine pools (DasSarma and DasSarma, 2015; Torregrosa-Crespo et al., 2017). Some halophiles are known to be polyextremophiles that are capable of tolerating and thriving not only in hypersaline environments, but also in settings with high pH, large amounts of sun radiation, and/or low water or nutrient availability. These harsh conditions have allowed halophiles to adapt unique

biochemical pathways of interest in both basic and applied research realms (Becker et al., 2014; Torregrosa-Crespo et al., 2017). Several products derived from these pathways are of particular commercial interest, including but not limited to: polyhydroxyalkanoates (plastics industry), amylases (biofuel industry), proteases (laundry detergent), beta-carotene (food additive), and glycerol (cosmetic industry) (Yin et al., 2015; Amoozegar et al., 2017).

The ability of halophiles to thrive in harsh conditions also makes them practical to use in the classroom. Because hypersaline growth conditions are inherently inhibitory to non-halophiles, halophilic culturing is more forgiving to small lapses in sterile technique, allowing students to successfully isolate pure cultures even if they are inexperienced in the laboratory. This is well-suited for introducing lab work and microbial culturing to first-year students. Although halophiles have a relatively long doubling time, a single weekly laboratory allows enough time for culture growth between each session.

Learning Outcomes and Course Overview

There are three main learning outcomes for the course: (1) students will be able to plan and perform the process of microbial culturing and genomic isolation, (2) students will be able to fit population growth models to microbial growth curve data, plot results, and compare the quality of fit among competing models, and (3) students will build interest and confidence in using quantitative skills in biology.

The structure of our 10-week quantitative biology CURE includes 1 weekly 3-h wet lab session, currently offered in the Molecular Prototyping and BioInnovation Lab at UC Davis, as well as a 1-h weekly lecture held in a traditional classroom. The lectures cover the quantitative theory associated with the hands-on research experience and connect this theory to the lab applications (**Supplementary File S2**). Students also complete a weekly homework that combines a lab write-up with quantitative problem-solving. As the course transitions into student projects, student learning is assessed with an initial project proposal, a final written report, and an oral group presentation.

The lab starts with an introduction to formal campus and site-specific lab safety training. We require all students to successfully complete the University of California "Fundamentals of Laboratory Safety Training," an online course required for everyone who works in labs on campus. We explain to the students that this training certifies them to work in faculty research labs on campus. The site-specific training highlights that the workspace is used outside of class hours to host active student research projects (i.e., they are working in a "real" research space and not just a classroom and thus that we expect them to be aware of those activities and associated hazards as they work). We emphasize this training to provide a solid foundation in safety, but also to establish a classroom environment in which students feel like they are doing authentic research.

Following the safety training, an introductory activity on pipetting, mixing, and measurement teaches techniques and orients students to the various instruments and supplies in the lab required for the course. These hands-on exercises are complemented with lecture and homework in which students develop their understanding of measurement and sources of error. For example, in one activity students repeatedly pipette and weigh an identical volume of solution. During lecture students had previously learned about accuracy and precision and how these can be quantified, and on the homework students use the programming language R (R Core Team, 2019) to calculate these quantities for their self-collected measurements. This pattern of linked lecture, lab, and homework continues throughout the quarter as each student collects (or selects previously collected) environmental samples, progresses through microbial isolation, and phenotypically characterizes the isolates.

In 2018, students worked with different table salts available from local supermarkets, as well as from environmental samples collected at the salt flats of Cabo Rojo, Puerto Rico. In 2019, students self-collected local samples from soil, water, or salty foods. Students then practice microbial culturing and isolation, using their samples to start cultures. For this course, we use Halobacterium medium 372 (DSMZ, 2007) as the base medium and vary the concentrations of NaCl. By using media with multiple salinities, we can potentially culture a broader diversity of microbes from the samples. All cultures are grown in either a shaking (liquid culture) or static (agar plate) incubator at 37°C. Plates that grow colonies within 7 days are transferred to a 4°C refrigerator to pause growth. To prepare pure cultures, students make phenotypic observations of a single colony of interest, select cells from the colony to inoculate a new liquid culture, and prepare microscope slides to make observations on cell size and shape using a standard compound microscope equipped with phase contrast optics.

With pure liquid cultures in hand, students focus on measuring and modeling microbial growth. Using a spectrophotometer, students measure the growth of each isolate at multiple salinities to quantitatively characterize salt tolerance. Students then use the programming language R (R Core Team, 2019) to fit logistic growth models to their data, explore variants of the models, and decide on the model that best explains the patterns they observe. The course culminates with students performing genomic DNA isolation and shipping off the gDNA for whole genome sequencing. These novel data are then used in a spring quarter follow-up CURE in which students continue to build quantitative skills as they apply bioinformatic and statistical techniques for comparative genomic analyses (**Figure 1**). **Supplementary Files S2–S7** present examples of additional curricular materials: a weekly course schedule, an example lab protocol, an R notebook for a computational lab, the associated RData file for the computational lab, the prompt outlining the students' final project, and an assignment that provides students with practice in writing up their final project report.

Iteration is considered a fundamental feature of CUREs. In this course, the steps described above may be iterated in practice by allowing students to revise and redo experimental or analytical steps. For instance, students have repeated opportunities to perform new isolations, select a new sample, redo growth measurements, and revise their quantitative growth models.

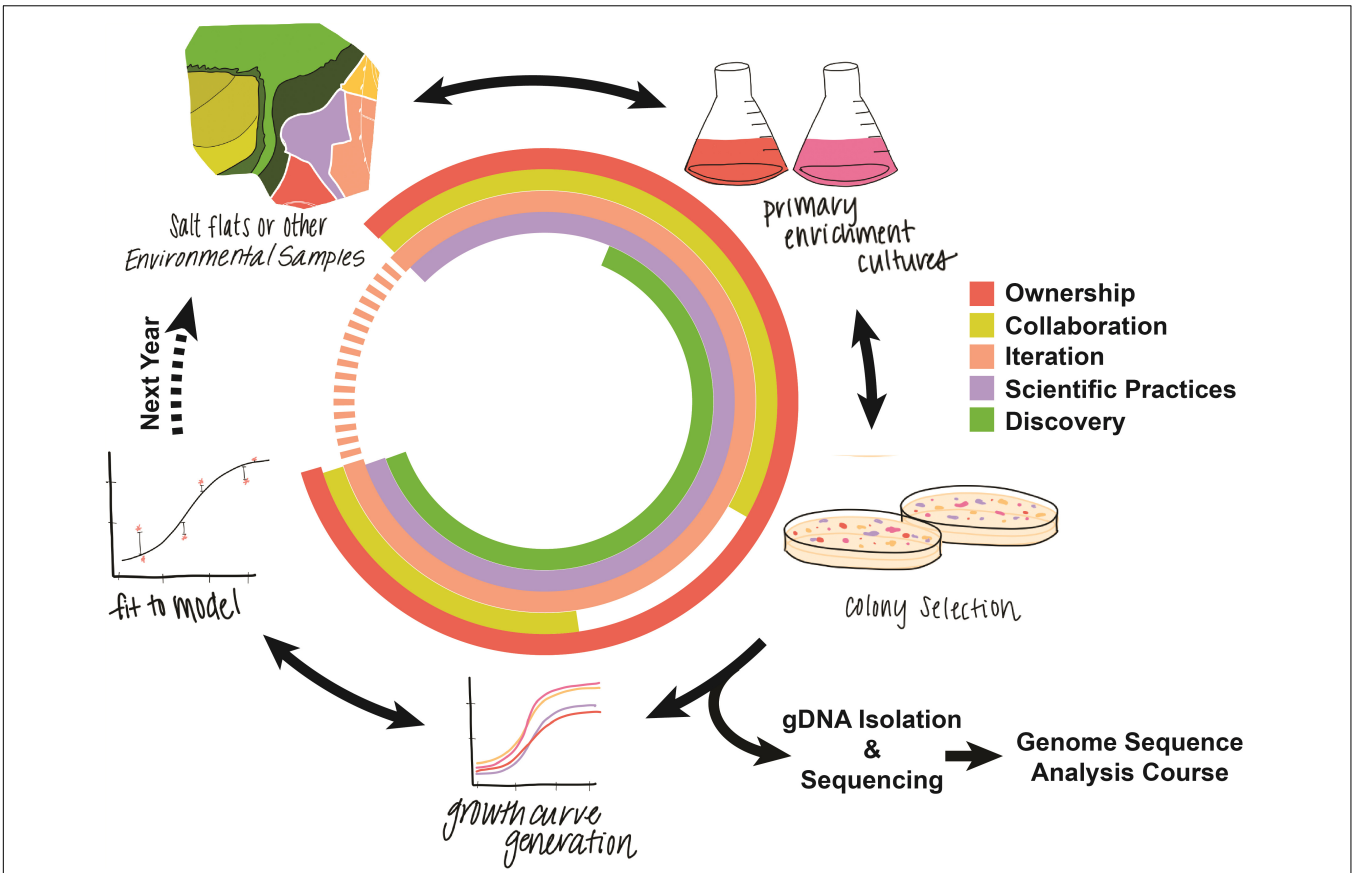

FIGURE 1 | Graphical overview of the course structure. Colors in the circle correspond to different core elements of course-based undergraduate research experiences. Arrows in both directions represent places where students can iteratively repeat and revise, for example re-plating primary enrichment cultures to look for novel colonies or fitting additional models to growth curves.

Figure 1 outlines the steps in the research process for this course, highlighting how the activities relate to iteration and other key features of CUREs, including peer collaboration, discovery, and scientific practices (Auchincloss et al., 2014).

We emphasize that students own every step of this project. They select their samples and media, maintain their cultures, and make their own decisions about how to revise their models. Because project ownership may be a critical mediator of students' overall benefits in CUREs (Corwin et al., 2018), the course aims to develop students into independent lab practitioners who are progressing their own projects.

Assessment of Student Attitudinal Gains

This work was approved by the University of California, Davis Institutional Review Board (protocol #1314250). We surveyed students who enrolled in the halophile CURE in the fall quarters of 2018 and 2019. Because this was an elective course that did not satisfy any major-specific requirements, this sample is likely biased toward students who are motivated to pursue laboratory and quantitative experience. As a basis for comparison, we recruited a non-overlapping group of survey respondents in the University Honors Program who had a biology-related major, both because many of our students were in the University Honors Program and because we hypothesized that other University Honors students may also be motivated to pursue research-oriented laboratory and quantitative experience. Some students who enrolled in the CURE were also in the University Honors Program (12 out of 16 students in 2018 and 5 out of 17 students in 2019). This comparison group was surveyed only in 2018, as the set of available comparison students in 2019 mostly overlapped with those from 2018.

Students completed an initial (pre) survey online during the first week of the quarter and completed an end-of-quarter (post) survey during the final weeks of the quarter. Students in the CURE completed the surveys during class time, while the comparison group completed their surveys at their own pace, outside of class. Authors JGA and MTF were co-instructors for the CURE in 2018, and authors JGA and REF were co-instructors in 2019. REF performed all data analysis on the anonymized student responses. The surveys contained multiple choice questions used in both years, and short response questions that were added in 2019.

Survey data can be contaminated by participants who provide inaccurate responses to questions. We ensured that students had read the questions by including one 5-point Likert-scale question that stated "We use this question to discard from the survey

TABLE 1 | The Math-Biology Values Instrument questions.

Item text	Construct
Using math to understand biology intrigues/would intrigue me.	Interest
It is/would be fun to use math to understand biology.	
Using math to understand biology appeals/would appeal to me.	
Using math to understand biology is/would be interesting to me.	
Math is valuable for me for my life science career.	Utility
It is important for me to be able to do math for my career in the life sciences.	
An understanding of math is essential for me for my life science career.	
Math will be useful to me in my life science career.	
I have/would have to work harder for a biology course that incorporates math than for one that does not.	Cost
I worry/would worry about getting worse grades in a biology course that incorporates math than one that does not.	
Taking a biology course that incorporates math intimidates/would intimidate me.	

All questions were framed with a 7-point Likert scale, asking "For each of the statements in this set please rate your agreement with the item in question." Respondents could answer "Strongly disagree," "Disagree," "Somewhat disagree," "Neither agree nor disagree," "Somewhat agree," "Agree," or "Strongly agree," which we translated to a 1 through 7 scale. These questions assess three underlying constructs, labeled Interest, Utility, and Cost.

people who are not reading the questions. Please select Agree (not Strongly agree) for this question to preserve your answers." We excluded from our analysis any survey responses with a choice other than "Agree" for this question for both the initial and end-quarter surveys. This excluded two students (out of 52 respondents). One additional student was excluded due to an incomplete survey.

Final Student Data

In total, we analyzed consenting, quality-controlled, paired pre and post responses from 16 CURE students in 2018 (94% of enrolled students), 17 CURE students in 2019 (77% of enrolled students), and 16 students in the comparison group (11% of the students initially emailed at the start of the quarter). Due to the small sample size of this study, we do not report specific demographic information or attempt to analyze the effect of demographics on student outcomes. Approximately 60% of students in both the CURE and comparison groups were female. 82% of students in the CURE were in their first or second year, as were all students in the comparison group. Student majors varied widely in both groups. Due to the range of years and majors, it is unlikely that a substantial proportion of either the CURE or comparison group shared any particular other courses. 17 of the 33 CURE students were in the University Honors Program, as were all 16 students in the comparison group. To have a larger sample size, data from both years of the CURE were merged and analyzed together. We note that patterns of student responses in the CURE group were similar in both years (**Supplementary Figure S1**). Our analysis focused on comparing survey responses on the end-of-quarter (post) survey to the responses on the initial (pre) survey, and contrasting these patterns between the CURE and comparison groups.

Student Attitudinal Changes

To assess changes in student attitudes, we used two previously created assessment instruments. The Math-Biology Values Instrument (**Table 1**; Andrews et al., 2017) assesses student attitudes toward using mathematics in biology, and is grounded in expectancy-value theory of achievement motivation and performance (Eccles et al., 1983; Wigfield and Eccles, 2000; Eccles and Wigfield, 2002). This theory posits that student achievement depends both on a student's confidence of success and the value they see in completing a task (Wigfield and Cambria, 2010; Corwin et al., 2018). This instrument consists of 11 questions that assess three underlying constructs related to student perceptions of using math in biology: ***interest*** in, ***utility*** of, and ***cost*** of taking biology courses that incorporate math (**Table 1**). To analyze student changes in their math-biology values, we created a subscore for each construct, averaging across all relevant questions. Finally, we used a Mann-Whitney *U*-test to compare the mean change in each subscore between the comparison group and the CURE students.

To evaluate student attitudes toward science learning and the scientific process, we used a subset of questions from the "Your opinions about yourself and about science" section from the CURE survey (Lopatto et al., 2008; Shaffer et al., 2010). The questions are summarized in **Table 2**. To avoid the problem of multiple comparisons that arise when testing the statistical significance of many individual questions, we relied on previous efforts that have assessed underlying relationships between questions. Prior analysis of this survey used factor analysis to identify the internal structure of these questions, finding two distinct constructs that are each assessed by multiple questions (Perera et al., 2017), and authors REF and MSG have noted similar correlations in student responses for these questions on

TABLE 2 | CURE Science Attitudes questions and their alignments with previously established constructs.

Item text	Construct
Even if I forget the facts, I'll still be able to use the thinking skills I learn in science.	Personal value
I get personal satisfaction when I solve a scientific problem by figuring it out myself.	
I can do well in science courses.	
Explaining science ideas to others has helped me understand the ideas better.	
There is too much emphasis in science classes on figuring things out for yourself.	Science Learning
I wish science instructors would just tell us what we need to know so we can learn it.	
Science is essentially an accumulation of facts, rules, and formulas.	
To be successful in biology, I need to be able to perform quantitative calculations.	*Calculations*
Mathematical models are useful for biology research.	*Models*

All questions were framed with a 5-point Likert scale, asking "For each of the statements in this set please rate your agreement with the item in question." Respondents could answer "Strongly disagree," "Disagree," "Neutral," "Agree," or "Strongly agree," which we translated to a 1 through 5 scale. Italicized items were newly created for this survey. Items in the Science Learning construct were reverse-scored for quantitative analysis, as they are negatively framed.

a different study of 1,800 student responses (Furrow, Caporale, and Goldman, unpublished). We created two subscores using the relevant questions from our survey and used Mann-Whitney *U*-tests to assess the statistical significance of differences among groups for each subscore. We label one construct Personal Value, following the nomenclature of Perera et al. (2017). The other construct is based on a smaller subset of the questions found to be correlated in prior work; because the questions all focus on science learning, we label the construct Science Learning. These questions are negatively framed, with greater disagreement expressing more positive attitudes toward science learning. Any quantitative analyses of student changes for this subscore are reverse-coded to assign higher values to more positive attitudes.

This section of the survey also included two additional statements posed in the same format (**Table 2**): "*Mathematical models are useful for biology research*" and "*To be successful in biology, I need to be able to perform quantitative calculations*," hereafter labeled as the Models and Calculations questions, respectively. These questions measure student perceptions of the utility of specific mathematical approaches in biology.

Changes in Students' Future Course and Career Plans

To assess students' future course and career plans related to quantitative biology, we asked additional qualitative questions in the survey for the 2019 iteration of the CURE. In the initial survey we asked: "*Do you have any plans to pursue future courses and/or a career in quantitative biology? Please briefly explain why or why not.*" In the end-of-quarter survey, we asked a matched short-answer question: "*Has this course changed your plans for pursuing future courses and/or a career in quantitative biology? If so, how?*" These end-of-quarter responses were then organized by theme (**Table 3**).

RESULTS

Math-Biology Values Had Minimal Change

Students taking the CURE did not differ significantly from the comparison students in the three constructs assessed by the Math-Biology Values Instrument (Andrews et al., 2017; *p*-values of 0.37, 0.41, and 0.78 for interest, utility, and cost, respectively). We note that both groups increased in both the perceived utility and cost of taking courses that include quantitative biology material (**Figures 2A,C**). However, it was difficult to assess changes in the Interest and Utility scores, especially for the CURE students, because even the start-of-quarter (pre-course) scores were near saturation (**Supplementary Figure S1**).

Gains in Student Attitudes Toward Learning Science

Students in the CURE had significantly more positive changes for the Learning Science construct ($p = 0.0009$). This appears to be largely driven by a more negative end-of-quarter response within the comparison group (**Figures 2B,D**). Although many CUREs have yielded positive attitudinal outcomes for students (e.g., Brownell et al., 2012, 2015; Jordan et al., 2014; Olimpo et al., 2016; Rodenbusch et al., 2016; Kirkpatrick et al., 2019; Murren et al., 2019), a pattern of more negative responses at the end of a course has been found in previous assessments of student perceptions of science (Adams et al., 2006; Semsar et al., 2011; Perera et al., 2017). Negative changes in attitudes may reflect the impact of other courses, as well as general changes in student morale toward the end of an academic term. Therefore, in the absence of a targeted educational intervention, in some cases the default expectation on a survey may be a slight decline in attitudes from the start to the end of a quarter. The CURE and comparison students did not differ significantly in their changes in Personal Value ($p = 0.63$), although this attribute was difficult to assess because both groups' pre-course responses were very positive (**Figures 2C,D**). For the two new questions about the utility of quantitative calculations and mathematical models, CURE students showed more positive changes only for the Calculations question, although neither comparison fell below a *p*-value of 0.05 (**Figures 2B,D**; $p = 0.051$ and $p = 0.36$ for Calculations and Models, respectively). Similar to the pattern for the Learning Science construct, students in the comparison group gave more negative responses about Calculations at the end of the quarter, while students in the CURE had a small average increase. However, we note that these mean changes in Calculations were small relative to the standard error for each mean. For the Models question, every respondent in both groups selected "Agree" or "Strongly agree" on both the initial and end-of-quarter survey. With such positive initial attitudes, there was limited opportunity for student responses to positively change and neither group showed much average change on this question (**Figure 2D**).

Some Students Increased Interest in Future Quantitative Experiences

Because students enrolled in the CURE had very positive initial attitudes toward quantitative biology, we added short-response questions in the 2019 survey for CURE students. We hoped that these would provide complementary insight into how student thinking and attitudes had changed as a result of completing the course. **Table 3** summarizes student responses to the initial (pre) question "*Do you have any plans to pursue future courses and/or a career in quantitative biology? Please briefly explain why or why not.*" and the end-of-quarter (post) question "*Has this course changed your plans for pursuing future courses and/or a career in quantitative biology? If so, how?*" These questions were only used in fall 2019 for students enrolled in the CURE course. Six of the 17 respondents expressed an increased interest in pursuing additional coursework or a career in quantitative biology. Two others shared that the course offered some useful clarification about what quantitative biology work entails. Five respondents had a sustained interest; these students expressed an interest in quantitative biology on the initial survey and did not indicate any change in their interest. Finally, four responses were not directly related to

TABLE 3 | Summary of student responses to the short-answer survey questions about future academic plans related to quantitative biology.

Theme (# students)	*[Pre] Do you have any plans to pursue future courses and/or a career in quantitative biology? Please briefly explain why or why not.*	*[Post] Has this course changed your plans for pursuing future courses and/or a career in quantitative biology? If so, how?*
Increased interest (6)	"As a biomedical engineer, I plan to focus on bioinformatics/bioimaging during my college career. Utilizing programming and mathematical modeling, I hope to gain more insight to human physiological processes while learning biology."	"I initially planned on pursuing just a major in Biomedical Engineering, but the content in this course has encouraged me to pursue a minor in Computational Biology. I sincerely enjoyed the mathematical applications related to biology and wish to pursue further research into mathematics in life sciences overall."
	"Yes! I am planning on pursuing a federal position as a government scientist in general scientific concerns, possibly, and most likely involving quantitative biology."	"This course has changed my plans career-wise in that I am much more comfortable considering a pursuit in quantitative biology."
	"Yes, quantitative biology is something that I have only recently learned more about and would definitely like to take more coursework if possible."	"I am definitely more inclined to investigating the possibilities that are available in quantitative biology. I was hoping to explore this interest further as I enrolled in this class and am happy to say that I definitely found the passion that I was expecting."
	"I'm not sure yet, but I wish to be a researcher in the future because it is interesting."	"Yes I might take more bio modeling courses"
	"I have no concrete plans, but I am taking this class to see whether such a career would interest me."	"I'm still undeclared and don't yet have any idea what my career will be. The course has definitely made me more interested in quantitative biology though. I will probably take BIS23B, and would consider taking more quantitative biology courses in the future."
	"Yes, because maths is making stuff more interesting and as a comp sci major i like maths	"it convinced me that quantitative biology is interesting"
Career clarification (2)	"I am unsure at the moment as I have never had to previously incorporate much mathematics in my biology courses before. I really enjoy learning and trying to understand the difficult concept in biology, but do find math quite intimidating. Though I know that math is fundamental for all science-related fields, I do not think I can see myself pursuing any careers in quantitative biology in the future."	"Overall, this course has helped me gain a better understanding as to how research and biology in certain experiments are best explained through the use of mathematical models. Though math is a challenging and often daunting subject for me, I do believe that it is essential to understanding and applying a bit of it into the science world. I [am] not sure if I can succeed in a career centered around quantitative biology."
	"While I'm not completely sure whether or not I will pursue a career in quantitative biology, I know for sure that I will continue taking quantitatively based biology classes and therefore I am pretty certain that I will most likely end up with a career in quantitative biology."	"It has definitely made me reconsider what exactly I want to do. I am still not sure about what I want to do or what to pursue in the future but this class has given me valuable insight."
Sustained interest (5)	"Yes as I am a genetics major and am wildly interested in going into research during my university career and beyond"	"I'm still interested in pursuing a career in genetics and genomics, this course has helped me basically see and understand the power of quantitative analysis in biology more so than I did before without experience"
	"I plan to pursue future courses in quantitative biology because biology is starting to become a data-driven science and it is important that undergraduates like myself are able to deal with this trend."	"I plan on taking BIS 23B and extra math courses to help understand other biological phenomena."
	"I am applying for graduate schools in the field of biomedical informatics and computational biology."	"No, I already planned to pursue a career in q bio"
	"Yes, it gives me a chance to apply what I know rather than soaking up information and not being able to do anything with it."	"No"
	"I would like to do the quantitative biology major because I am interested in both cs and biology"	"After taking this course, I would like to take BIS 23B in the spring (and perhaps BIS 20Q in the winter)."
Unclear (4)	"Maybe. I'm thinking about researching epigenetics for medical applications. I know that bioinformatics is important and that big data is becoming more prevalent in genetics. I would say quantitative biology isn't my goal, but may be where I end up."	"Honestly, the coding component was kind of a shock. It's tough at first but rewarding once you finally get it."
	"Yes, I plan to work in a research field in Genetics."	"I really wanted to take a class that gave me an experience of what a lab actually is like, this class did that and was really enjoyable."
	"I am unsure of whether I want to pursue future courses and/or a career in quantitative biology because I am still unsure of what such courses/careers would entail."	"It hasn't really changed much of my plans."
	"At the moment, no. I took this course to gain more lab experience."	"Plan on doing research"

Student pre and post responses are aligned by row. The initial (pre) survey asked students about future plans, and the end-of-quarter (post) survey asked if their plans had changed. These questions were asked only to the CURE students enrolled in 2019 (17 respondents in total). We categorized the paired pre and post responses into four codes: "Increased interest," "Career clarification," "Sustained interest," and "Unclear." Responses that directly mentioned an increase in confidence or interest in future courses or careers in quantitative biology were coded "Increased interest." Responses that discussed insight or understanding about what this work looks like were coded "Career clarification." The "Sustained interest" code was used for responses that didn't explicitly mention any gain in interest, but expressed similarly positive plans in both pre and post. Responses that did not clearly address the survey questions were coded as "Unclear."

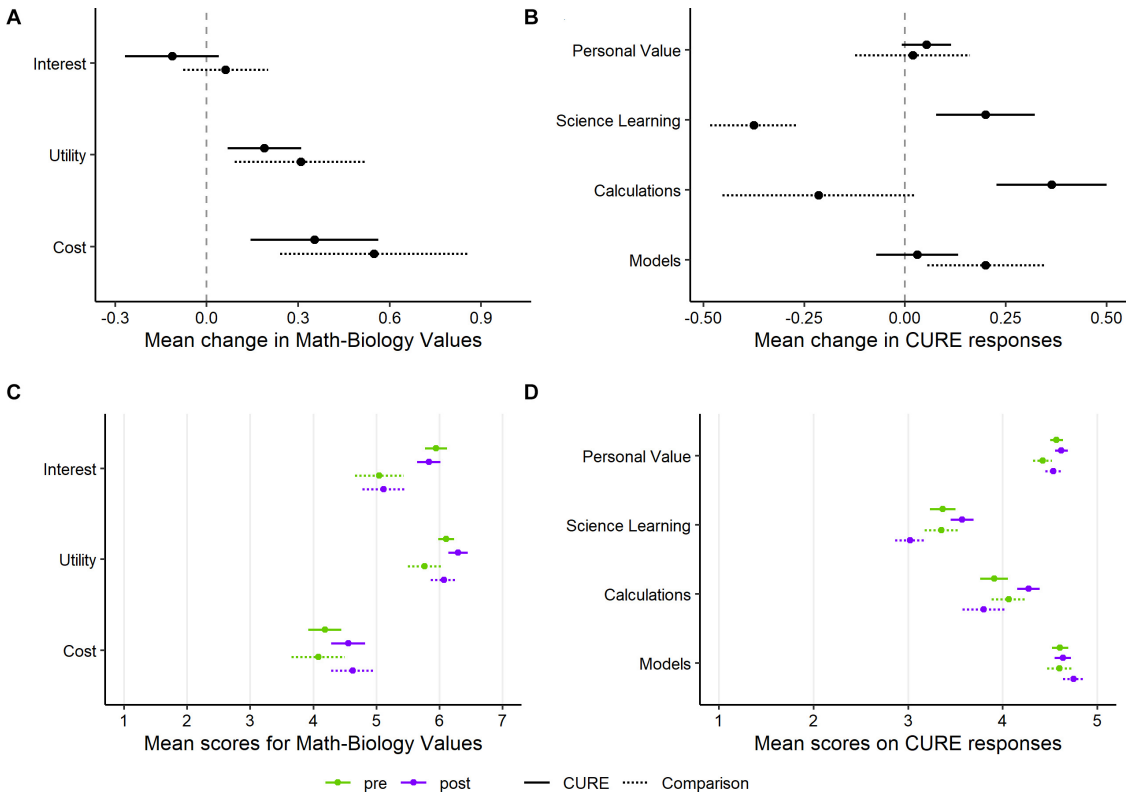

FIGURE 2 | Mean change (post score minus pre score) in student response between initial (pre) and end-of-quarter (post) survey, as well as mean pre and post scores, for several assessment instruments. **(A)** Change for each of the three constructs assessed by the Math-Biology Values Instrument, averaged over the questions for each construct. **(B)** Change for two constructs assessed by questions on the CURE survey, as well as two additional questions specific to quantitative thinking (Calculations, Models). **(C)** Initial (pre) and end-of-quarter (post) scores for the Math-Biology Values Instrument. **(D)** Initial (pre) and end-of-quarter (post) scores for CURE survey, Calculations, and Models. Students in both the CURE and comparison groups had very high initial scores for Interest, Utility, Personal Value, and Models, limiting the potential to observe positive changes. Green indicates the initial (pre) survey scores and purple indicates the end-of-quarter (post) scores. Points show the mean and lines show ± the standard error of the mean (SEM) within each group, question, and survey timing. Solid lines show SEM for CURE students, and dotted lines show SEM for comparison students. There are 33 students in the CURE group and 16 students in the comparison group.

the prompts or could not readily be placed into the categories mentioned above.

DISCUSSION

We have outlined an introductory course-based undergraduate research experience that focuses on building students' practical laboratory technique and developing quantitative skills for mathematical modeling. Initial assessment of student attitudes during the first 2 years of this course suggest that, relative to a comparison group, students develop more positive attitudes toward the process of learning science, and potentially also see more value to using quantitative calculations in biology (**Figure 2**). More than one-third of respondents in 2019 also expressed greater interest in taking additional quantitative courses or pursuing future work in quantitative biology (**Table 3**).

Changes in students' future quantitative biology course and career plans seemed to be driven in part by an increased interest in or enjoyment of quantitative biology. Among the six students who mentioned a change in their plans, two explicitly discussed increased interest as a factor shaping their future plans (e.g., "The course has definitely made me more interested in quantitative biology.") and two others mentioned positive feelings about doing quantitative biology (e.g., "I definitely found the passion that I was expecting."). Some responses also revealed how students might be weighing the relative utility and cost of learning quantitative biology (e.g., "Though math is a challenging and often daunting subject for me, I do believe that it is essential to understanding and applying a bit of it into the science world. . ."). In future course implementations, post-course student interviews might help reveal how different dimensions of these attitudes interact to shape students' future academic decision-making.

The Likert-scale assessment of values surrounding the role of math in biology found limited evidence of gains. However, the students enrolled in this course entered with high interest and already believed that quantitative skills were useful in biology, as reflected in the high pre-course scores in the categories of Interest, Utility, Personal Value, and Models (**Supplementary Figure S1**). This high baseline limited the potential for us to identify gains in these affectual categories.

At the end of the quarter, students in both the CURE and the comparison group perceived a higher cost (e.g., higher workload or lower grades) to taking biology courses that incorporate math. This might be expected, as even students who have a positive, confidence-building experience may develop more realistic expectations about the potential challenges ahead. However, a student's belief in their ability to succeed at an academic task can affect their academic achievement (Doménech-Betoret et al., 2017), so high perceptions of cost may negatively impact a student's course outcomes. As we assess larger sample sizes of students who complete both this fall course in microbial culturing as well as the quantitative spring course in comparative genomics, we plan to analyze whether the longer experience over two quarters might produce shifts toward lower perceived costs. Previous work assessing the Genomics Education Partnership CURE (Shaffer et al., 2010) suggests that students perceive greater learning benefits from longer experiences working on their research projects (Shaffer et al., 2014).

In future versions of this course, we hope to include additional activities focused on helping students build their identity as scientists. By design, the course's focus on student research implicitly places students in the role of research scientists. However, student scientific identity could potentially be developed more explicitly by diversifying the structure of meetings and assignments to promote the kind of informal critical thinking, curiosity, and collaboration that occurs in research labs. Examples might include journal clubs, science coffee chats, poster making and presenting, and academic writing practice. To expand collaboration, one might convert part of each lab into a "lab meeting" in which students discuss with peers and take turns summarizing primary literature or sharing updates on their research (e.g., in the CURE presented by Oufiero, 2019). In addition, we would like to help students understand that failure, mistakes, and repeated iteration of data gathering and analysis are normal parts of scientific inquiry. Although the instructors in this CURE discussed these themes in passing during lecture and lab (Seidel et al., 2015), these goals were not explicit in our lesson planning or assessments.

One logistical challenge for this course was the organization of isolate metadata. Student project ownership may be a foundational source of student benefits from CUREs (Corwin et al., 2018), so we asked students to own their data from sample to final isolate to quantitative growth data. After students struggled to maintain a complete chain of metadata for their samples in 2018, we implemented a Google Sheets system in which all new data were added as new columns to a continually growing classroom document. This became cumbersome by the end of the quarter, but also provided a shared workspace in which students could note each other's discoveries and feel like a part of a team effort.

Although we focused on salt-dependent growth rates for halophiles, this course could be adapted to other CUREs based around alternative research questions. Other phenotypic investigations might include color production, sugar utilization, halophilic gas vesicle production, or the production of easy-to-spot products like polyhydroxyalkanoates—all of which require a separate set of research techniques scalable to a course timeframe (e.g., colorimetric assays, visible light microscopy, etc.). One could expand on this to culture strains in various growth conditions such as shaking, oxygenation, salinity, pH, nutrient availability, and/or temperature. The most promising alternatives are likely to be those which use a highly selective growth medium, preventing the cultures from being swamped by local contaminants. Our high-salinity media helped reduce contamination problems; extreme temperature, pH, or unusual food sources might be similarly effective.

The skills developed and data created from microbial culturing provide a productive way to engage undergraduate students in course-based research. By combining laboratory skills with growth rate modeling, students learn quantitative skills in a low-stakes environment in which they have ownership over the data they are generating and analyzing. The current version of this quantitative biology CURE emphasizes the growth rates of halophilic microbes, but we expect that this model for course design and implementation can be readily applied to a broad range of organisms and phenotypes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

AY, KD, HK, JE, JA, MG, MF, RF, and SA contributed to conception and design of the course. MG, MF, and RF contributed to conception and design of the educational assessment. JA, MF, and RF each served as co-instructors for at least one iteration of the course. HK and SA each served as teaching assistants for one iteration of the course. AY provided technical laboratory support. RF performed the qualitative and statistical analysis. KD, HK, MF, RF, and SA wrote the initial draft of the manuscript. HK, MF, MG, and RF reviewed and edited the initial draft.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Adams, W. K., Perkins, K. K., Podolefsky, N. S., Dubson, M., Finkelstein, N. D., and Wieman, C. E. (2006). New instrument for measuring student beliefs about physics and learning physics: the colorado learning attitudes about science survey. *Phys. Rev. Spec. Top.* 2:010101. doi: 10.1103/PhysRevSTPER.2.010101

Agustin, M. Z. N., and Agustin, M. A. (2009). Algebra and precalculus skills and performance in first-semester calculus. *Int. J. Case Method Res. Appl.* 21, 232–236.

Aikens, M. L., and Dolan, E. L. (2014). Teaching quantitative biology: goals, assessments, and resources. *Mol. Biol. Cell* 25, 3478–3481. doi: 10.1091/mbc.e14-06-1045

American Association for the Advancement of Science (2011). *Vision and Change in Undergraduate Biology Education: A Call to Action*. Washington, DC: American Association for the Advancement of Science.

Amoozegar, M. A., Siroosi, M., Atashgahi, S., Smidt, H., and Ventosa, A. (2017). Systematics of haloarchaea and biotechnological potential of their hydrolytic enzymes. *Microbiology* 163, 623–645. doi: 10.1099/mic.0.000463

Andrews, S. E., Runyon, C., and Aikens, M. L. (2017). The math-biology values instrument: development of a tool to measure life science majors' task values of using math in the context of biology. *CBE Life Sci. Educ.* 16:ar45. doi: 10.1187/cbe.17-03-0043

Auchincloss, L. C., Laursen, S. L., Branchaw, J. L., Eagan, K., Graham, M., Hanauer, D. I., et al. (2014). Assessment of course-based undergraduate research experiences: a meeting report. *CBE Life Sci. Educ.* 13, 29–40. doi: 10.1187/cbe.14-01-0004

Bangera, G., and Brownell, S. E. (2014). Course-based undergraduate research experiences can make scientific research more inclusive. *CBE Life Sci. Educ.* 13, 602–606. doi: 10.1187/cbe.14-06-0099

Becker, E. A., Seitzer, P. M., Tritt, A., Larsen, D., Krusor, M., Yao, A. I., et al. (2014). Phylogenetically driven sequencing of extremely halophilic archaea reveals strategies for static and dynamic osmo-response. *PLoS Genet.* 10:e1004784. doi: 10.1371/journal.pgen.1004784

Bialek, W., and Botstein, D. (2004). Introductory science and mathematics education for 21st-century biologists. *Science* 303:788. doi: 10.1126/science.1095480

Brownell, S. E., Hekmat-Scafe, D. S., Singla, V., Chandler Seawell, P., Conklin Imam, J. F., Eddy, S. L., et al. (2015). A high-enrollment course-based undergraduate research experience improves student conceptions of scientific thinking and ability to interpret data. *CBE Life Sci. Educ.* 14:ar21. doi: 10.1187/cbe.14-05-0092

Brownell, S. E., Kloser, M. J., Fukami, T., and Shavelson, R. (2012). Undergraduate biology lab courses: comparing the impact of traditionally based "cookbook" and authentic research-based courses on student lab experiences. *J. Coll. Sci. Teach.* 41, 36–45.

Chiel, H. J., McManus, J. M., and Shaw, K. M. (2010). From biology to mathematical models and back: teaching modeling to biology students, and biology to math and engineering students. *CBE Life Sci. Educ.* 9, 248–265. doi: 10.1187/cbe.10-03-0022

Coil, D. (2017). *Koala Poop Microbiome Class: Summary and Class Materials*. Available online at: https://microbe.net/2017/01/09/koala-poop-microbiome-class-summary-and-class-materials/ (accessed June 23, 2020).

Corwin, L. A., Runyon, C. R., Ghanem, E., Sandy, M., Clark, G., Palmer, G. C., et al. (2018). Effects of discovery, iteration, and collaboration in laboratory courses on undergraduates' research career intentions fully mediated by student ownership. *CBE Life Sci. Educ.* 17:ar20. doi: 10.1187/cbe.17-07-0141

Dahlhausen, K. (2018). *Nectar CURE: Week 1*. Available online at: https://microbe.net/2018/04/11/nectar-cure-week-1/ (accessed June 23, 2020).

DasSarma, S., and DasSarma, P. (2015). Halophiles and their enzymes: negativity put to good use. *Curr. Opin. Microbiol.* 25, 120–126. doi: 10.1016/j.mib.2015.05.009

Depelteau, A. M., Joplin, K. H., Govett, A., Miller, H. A., and Seier, E. (2010). SYMBIOSIS: development, implementation, and assessment of a model curriculum across biology and mathematics at the introductory level. *CBE Life Sci. Educ.* 9, 342–347. doi: 10.1187/cbe.10-05-0071

Dolan, E. L. (2016). *Course-based Undergraduate Research Experiences: Current knowledge and future directions*. Washington, DC: National Research Council.

Doménech-Betoret, F., Abellán-Roselló, L., and Gómez-Artiga, A. (2017). Self-efficacy, satisfaction, and academic achievement: the mediator role of students' expectancy-value beliefs. *Front. Psychol.* 8:1193. doi: 10.3389/fpsyg.2017.01193

DSMZ (2007). *Recipe for Halobacterium Medium 372*. Available online at: https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium372.pdf (accessed January 14, 2020).

Duffus, D., and Olifer, A. (2010). Introductory life science mathematics and quantitative neuroscience courses. *CBE Life Sci. Educ.* 9, 370–377. doi: 10.1187/cbe.10-03-0026

Dunitz, M. I., Lang, J. M., Jospin, G., Darling, A. E., Eisen, J. A., and Coil, D. A. (2015). Swabs to genomes: a comprehensive workflow. *PeerJ* 3:e960. doi: 10.7717/peerj.960

Eaton, C. D., and Highlander, H. C. (2017). The case for biocalculus: design, retention, and student performance. *CBE Life Sci. Educ.* 16:ar25. doi: 10.1187/cbe.15-04-0096

Eccles, J., Adler, T., Futterman, R., Goff, S., Kaczala, C., Meece, J., et al. (1983). "Expectancies, values, and academic behaviors," in *Achievement and Achievement Approaches*, ed. J. T. Spence (San Francisco, CA: W. H. Freeman), 75–146.

Eccles, J. S., and Wigfield, A. (2002). Motivational beliefs, values, and goals. *Annu. Rev. Psychol.* 53, 109–132. doi: 10.1146/annurev.psych.53.100901.135153

Ellis, J., Fosdick, B. K., and Rasmussen, C. (2016). Women 1.5 times more likely to leave STEM pipeline after calculus compared to men: lack of mathematical confidence a potential culprit. *PLoS One* 11:e0157447. doi: 10.1371/journal.pone.0157447

Jordan, T. C., Burnett, S. H., Carson, S., Caruso, S. M., Clase, K., DeJong, R. J., et al. (2014). A broadly implementable research course in phage discovery and genomics for first-year undergraduate students. *MBio* 5:e01051-13. doi: 10.1128/mBio.01051-13

Kirkpatrick, C., Schuchardt, A., Baltz, D., and Cotner, S. (2019). Computer-based and bench-based undergraduate research experiences produce similar attitudinal outcomes. *CBE Life Sci. Educ.* 18:ar10. doi: 10.1187/cbe.18-07-0112

Lopatto, D., Alvarez, C., Barnard, D., Chandrasekaran, C., Chung, H.-M., Du, C., et al. (2008). Genomics education partnership. *Science* 322, 684–685. doi: 10.1126/science.1165351

Ma, X., and Kishor, N. (1997). Assessing the relationship between attitude toward mathematics and achievement in mathematics: a meta-analysis. *J. Res. Math. Educ.* 28, 26–47. doi: 10.2307/749662

Miller, J. E., and Walston, T. (2010). Interdisciplinary training in mathematical biology through team-based undergraduate research and courses. *CBE Life Sci. Educ.* 9, 284–289. doi: 10.1187/cbe.10-03-0046

Murren, C. J., Wolyniak, M. J., Rutter, M. T., Bisner, A. M., Callahan, H. S., Strand, A. E., et al. (2019). Undergraduates phenotyping arabidopsis knockouts in a course-based undergraduate research experience: exploring plant fitness and vigor using quantitative phenotyping methods. *J. Microbiol. Biol. Educ.* 20. doi: 10.1128/jmbe.v20i2.1650 [Epub ahead of print].

National Research Council (2003). *BIO2010: Transforming Undergraduate Education for Future Research Biologists*. Washington, DC: National Research Council. doi: 10.17226/10497

National Research Council (2009). *A New Biology for the 21st Century*. Washington, DC: National Research Council. doi: 10.17226/12764

Olimpo, J. T., Fisher, G. R., and DeChenne-Peters, S. E. (2016). Development and evaluation of the tigriopus course-based undergraduate research experience: impacts on students' content knowledge, attitudes, and motivation in a majors introductory biology course. *CBE Life Sci. Educ.* 15:ar72. doi: 10.1187/cbe.15-11-0228

Oufiero, C. E. (2019). The organismal form and function lab-course: a new CURE for a lack of authentic research experiences in organismal biology. *Integr. Org. Biol.* 1:obz021. doi: 10.1093/iob/obz021

Perera, V., Mead, C., Buxner, S., Lopatto, D., Horodyskyj, L., Semken, S., et al. (2017). Students in fully online programs report more positive attitudes toward science than students in traditional, in-person programs. *CBE Life Sci. Educ.* 16:ar60. doi: 10.1187/cbe.16-11-0316

R Core Team (2019). *R: A Language and Environment for Statistical Computing (Version 3.6.1)*. Vienna: R Foundation for Statistical Computing.

Rodenbusch, S. E., Hernandez, P. R., Simmons, S. L., and Dolan, E. L. (2016). Early engagement in course-based research increases graduation rates and completion of science, engineering, and mathematics degrees. *CBE Life Sci. Educ.* 15:ar20. doi: 10.1187/cbe.16-03-0117

Rodriguez-Medina, J., Kim, H. G., Castro, J., Contreras, C. M., Glon, C. L., Goyal, A., et al. (2020). Draft genome sequences of 16 halophilic prokaryotes isolated from diverse environments. *Microbiol. Resour. Announc.* 9:e01540-19. doi: 10.1128/MRA.01540-19

Seidel, S. B., Reggi, A. L., Schinske, J. N., Burrus, L. W., and Tanner, K. D. (2015). Beyond the biology: a systematic investigation of noncontent instructor talk in an introductory biology course. *CBE Life Sci. Educ.* 14:ar43. doi: 10.1187/cbe.15-03-0049

Semsar, K., Knight, J. K., Birol, G., and Smith, M. K. (2011). The colorado learning attitudes about science survey (CLASS) for use in biology. *CBE Life Sci. Educ.* 10, 268–278. doi: 10.1187/cbe.10-10-0133

Shaffer, C. D., Alvarez, C., Bailey, C., Barnard, D., Bhalla, S., Chandrasekaran, C., et al. (2010). The genomics education partnership: successful integration of research into laboratory classes at a diverse group of undergraduate institutions. *CBE Life Sci. Educ.* 9, 55–69. doi: 10.1187/09-11-0087

Shaffer, C. D., Alvarez, C. J., Bednarski, A. E., Dunbar, D., Goodman, A. L., Reinke, C., et al. (2014). A course-based research experience: how benefits change with increased investment in instructional time. *CBE Life Sci. Educ.* 13, 111–130. doi: 10.1187/cbe-13-08-0152

Sonnert, G., and Sadler, P. M. (2014). The impact of taking a college pre-calculus course on students' college calculus performance. *Int. J. Math. Educ. Sci. Technol.* 45, 1188–1207. doi: 10.1080/0020739X.2014.920532

Torregrosa-Crespo, J., Galiana, C. P., and Martínez-Espinosa, R. M. (2017). "Biocompounds from haloarchaea and their uses in biotechnology," in *Archaea – New Biocatalysts, Novel Pharmaceuticals and Various Biotechnological Applications*, eds H. Sghaier, A. Najjari, and K. Ghedira (London: IntechOpen), doi: 10.5772/intechopen.69944

Treisman, U. (1992). Studying students studying calculus: a look at the lives of minority mathematics students in college. *Coll. Math. J.* 23, 362–372. doi: 10.1080/07468342.1992.11973486

Vater, A., Agbonavbare, V., Carlin, D. A., Carruthers, G. M., Chac, A., Doroud, L., et al. (2016). Draft genome sequences of *Shewanella* sp. Strain UCD-FRSP16_17 and nine vibrio strains isolated from abalone feces. *Genome Announc.* 4:e00977-16. doi: 10.1128/genomeA.00977-16

Vater, A., Dahlhausen, K., Coil, D. A., Anderton, B. N., Wirawan, C. S., Caporale, N., et al. (2019). First-year seminars as a venue for course-based undergraduate research experiences: a preliminary report. *Bioscene* 45, 3–10.

Wachsmuth, L. P., Runyon, C. R., Drake, J. M., and Dolan, E. L. (2017). Do biology students really hate math? Empirical insights into undergraduate life science majors' emotions about mathematics. *CBE Life Sci. Educ.* 16:ar49. doi: 10.1187/cbe.16-08-0248

Wigfield, A., and Cambria, J. (2010). Students' achievement values, goal orientations, and interest: definitions, development, and relations to achievement outcomes. *Dev. Rev.* 30, 1–35. doi: 10.1016/j.dr.2009.12.001

Wigfield, A., and Eccles, J. S. (2000). Expectancy-value theory of achievement motivation. *Contemp. Educ. Psychol.* 25, 68–81. doi: 10.1006/ceps.1999.1015

Yin, J., Chen, J.-C., Wu, Q., and Chen, G.-Q. (2015). Halophiles, coming stars for industrial biotechnology. *Biotechnol. Adv.* 33, 1433–1442. doi: 10.1016/j.biotechadv.2014.10.008

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REVIEW
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Microbiomes for All

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Microbiome research projects are often interdisciplinary, involving fields such as microbiology, genetics, ecology, evolution, bioinformatics, and statistics. These research projects can be an excellent fit for undergraduate courses ranging from introductory biology labs to upper-level capstone courses. Microbiome research projects can attract the interest of students majoring in health and medical sciences, environmental sciences, and agriculture, and there are meaningful ties to real-world issues relating to human health, climate change, and environmental sustainability and resilience in pristine, fragile ecosystems to bustling urban centers. In this review, we will discuss the potential of microbiome research integrated into classes using a number of different modalities. Our experience scaling-up and implementing microbiome projects at a range of institutions across the US has provided us with insight and strategies for what works well and how to diminish common hurdles that are encountered when implementing undergraduate microbiome research projects. We will discuss how course-based microbiome research can be leveraged to help faculty make advances in their own research and professional development and the resources that are available to support faculty interested in integrating microbiome research into their courses.

Keywords: undergraduate research, microbiology education, big data, data analysis, microbiomes, course-based undergraduate research

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INTRODUCTION

The study of microbiomes has skyrocketed over the last decade and has advanced our understanding of human health and disease, complex ecological systems, microbial diversity, and evolution (Falkowski et al., 2008; Locey and Lennon, 2016; Thompson et al., 2017; Almeida et al., 2019). The use of the term, microbiome, has jumped from fewer than 5 publications/year prior to 2008, to more than 6,000 publications/year in 2019, and microbiome studies have been the focus of numerous news and internet stories (Bik, 2016; Schmulson and Bashashati, 2018; Abid, 2019). Growth in microbiome research has been driven in part by new DNA and RNA sequencing and analysis technologies, and by a paradigm shift in the field of microbial ecology, sparked by culture-independent techniques (we will use culture-independent to include both metagenomics, *sensu stricto*, and gene-targeted amplicon sequencing) (Handelsman, 2004; Riesenfeld et al., 2004; Escobar-Zepeda et al., 2015; Goodwin et al., 2016; Slatko et al., 2018). These changes created an opportunity to bring the excitement and potential of microbiome studies to students through training in the scientific process and their engagement in research (Jurkowski et al., 2007; National Research Council, 2007). This review discusses microbiome research in teaching microbiology to students at two intersections with the real-world, (1) the ability to advance understanding in areas of human health and disease, biodiversity, evolution, biotechnology, climate science, and other fields, and (2) the increasingly in-demand skills of quantitative reasoning, statistics, and data skills

(mining, analysis, interpretation and visualization), and the spectrum of STEM classroom and laboratory contexts in which students receive their training. As a target of exploration in STEM education, microbiomes capture our imagination with their complexity, ubiquity, and potential to contribute solutions to global health and environmental crises (Blaser et al., 2016; Finbow, 2019). For educators, the versatility of microbiome studies as a scaffold for teaching microbiology, ecology, evolution, genetics, bioinformatics, and data analysis, is unmatched.

Microbiome research projects are ideal for teaching microbiology in a real-world context. Importantly, large microbiome data sets can be generated and analyzed in a massively parallel fashion by students working individually or in small groups (Hingamp et al., 2008; Boyle, 2010; Buonaccorsi et al., 2011; Bolyen et al., 2019). Students are excited by work on unanswered questions and take ownership of research projects that make use of samples they have collected in their communities and local environments (Lopatto, 2010; Hanauer and Dolan, 2014; Weber et al., 2015; Cooper et al., 2019a). Culture-independent approaches do not require growing microorganisms in the lab. As a result, this work poses few safety risks to students and allows microbiome research in almost any classroom setting and expands the reach of these research projects to citizen science initiatives (Freeman et al., 2016; Handelsman et al., 2018; American Society for Microbiology, 2019; Genné-Bacon and Bascom-Slack, 2019; Basalla et al., 2020). Because of the flexibility and the range of questions that can be addressed, microbiome studies open avenues to interdisciplinary research that extend across courses, departments, and institutions. The instrumentation required for culture-independent studies of microbial diversity does not need to be extensive, making these projects accessible to many high school, community college, and public university faculty (Estes, 2015; Robertson-Albertyn, 2016). Investigating and analyzing microbiome data is ideal for training in quantitative reasoning, data analysis, and data presentation. While this list of strengths associated with implementing student microbiome projects is significant, there are also significant hurdles, and these vary depending on the background and expertise of faculty and the availability of resources. For those who have experience working with microbiomes, classroom and laboratory logistics and pedagogical considerations remain a primary challenge. For faculty that are veterans of undergraduate research experiences (UREs) and course-based research experiences (CUREs), but who are new to research into microbiomes, the fast-paced advances in sequencing and data analyses tools can be a challenge to keep up with and add an element of uncertainly to implementing microbiome projects.

In the literature searches for this review we found more than twenty published examples of undergraduate microbiome research projects (**Table 1**) that may serve as helpful aids for those looking for guidance on designing and structuring a course that includes microbiome research. The review gives examples of how the challenges of experimental design, data collection, and data analysis with students have been addressed by others. There are communities of faculty with experience in undergraduate microbiome research projects, such as the Research Experiences in Microbiomes Network (REMNet, an NSF RCN-UBE), and communities such as these can be an additional source for ideas and support for developing student microbiome research projects. Using a range of UREs, including CUREs, guided inquiry, capstone research projects, intensive summer research experiences, and other modalities, we and others have developed resources for faculty and students to explore the diversity and complexity of their local environments using microbiome research projects. The tools for studying microbial communities, and for DNA or RNA sequencing and data analysis, are increasingly accessible and affordable, and they can extend the reach of UREs into cutting-edge applications. Moreover, hands-on experiences addressing real-world questions are an important part of training of the next generation of STEM professionals for a society where complex scientific and technological skills will be critical (Kloser et al., 2011; Vision and Change in Undergraduate Biology Education, 2011; Auchincloss et al., 2014).

ADVANCING SCIENTIFIC KNOWLEDGE THROUGH MICROBIOME RESEARCH

The opportunity for significant impacts from microbiome research has been made possible by the confluence of emerging forces – the culture-independent study of microbial communities, the power and accessibility of next-generation DNA sequencing and analysis tools, and the push to provide research experiences to more students (Handelsman, 2004; Escobar-Zepeda et al., 2015). The pioneering work on 16S rRNA genes by Carl Woese and the initial culture-independent studies of bacteria in the mid-80s and 90s by Norman Pace, Jo Handelsman and others, have invigorated and revolutionized the field of microbiology (Gutell et al., 1994; Riesenfeld et al., 2004; Frank and Pace, 2008). Prior to these breakthroughs, little of the microbial world could be coaxed to grow under laboratory conditions, and often less than 1% of the diversity from samples could be studied. This was hinted at by the discrepancy between the diversity of bacteria seen by microscopy and the relative lack of diversity in what could be cultured in the lab from the same sample (the "great plate count anomaly," Staley and Konopka, 1985). Culture-independent approaches, which use the extractable DNA as a proxy for the microorganisms present in a sample, now allow investigators to routinely study > 95% of the diversity in a sample – thus opening up new paths of discovery and insight into how the world's most numerous and influential cells (and viruses) are shaping the environment (Blaser et al., 2016). The advances have a far-reaching impact on critical research and development areas, including drug discovery, agriculture, environmental sustainability, and ecosystem resilience in the face of anthropogenic forces such as urbanization and climate change (Nielsen et al., 2012; King, 2014; Barea, 2015; Harvey et al., 2015; Jez et al., 2016; Brown and Hazen, 2017; Krüger et al., 2018; van de Guchte et al., 2018; Podolsky et al., 2019; Xie et al., 2019).

Following on the heels of culture-independent approaches to studying microbiomes, the first wave of next-generation DNA

TABLE 1 | Microbiome research projects for undergraduate students.

References	Topic	Audience	Discipline
Boomer et al., 2002	Green non-sulfur bacteria of Yellowstone NP	Upper-level UG	Molecular biology
Hingamp et al., 2008	Metagenome annotation	Upper-level UG	Cell biology and Biochemistry
Rios-Velazquez et al., 2011	Soil microbiomes	Upper-level UG	Microbiology
Donato et al., 2012	Identification of a reductase gene	Upper-level UG	Biochemistry
Edwards et al., 2013	Marine microbiomes	Upper-level UG	Ecology, multidisciplinary
Muth and McEntee, 2014	Urban microbiomes	Introductory and upper-level UG	Microbiology, Intro-biology
Rundell et al., 2014	Winogradsky columns	NS	Microbiology
Sanders et al., 2016	Plant microbiomes	Upper-level UG	Biology
Docherty et al., 2015	Soil microbiomes	NS	Multidisciplinary
Gibbens et al., 2015	Mississippi River water samples	Introductory UG	Biology
Muterspaw et al., 2015	Mixed environmental samples	Upper-level UG	Ecology, Computer Science
Wang, 2017	Oral microbiomes	Upper-level UG	Biology, Biotechnology, Microbiology
Hartman et al., 2016	Personal microbiomes	Introductory UG	Bioinformatics
Coil et al., 2017	Gut microbiome board game	Introductory UG	Microbiology
Costas et al., 2017	Influence of fertilizer on nitrogen-fixing microorganisms	Introductory HS	Biology (HS)
Hotaling et al., 2018	Prokaryotic diversity	Introductory UG	Biology
Lentz et al., 2017	Human umbilicus microbiomes	Introductory and upper-level UG	Biology and Biotechnology
Stevens et al., 2017	Environmental microbial communities	Upper-level UG	Microbiology
Alessi et al., 2018	Soil microbiomes using isolation chips (iChips)	Upper-level UG/MS	Molecular microbial ecology
Scott Weber et al., 2018	Personal microbiomes	Upper-level UG	Immunology, Molecular Biology, Genomics
Tobin and Shade, 2018	Effect of temperature on soil bacteria	Upper-level UG	Microbiology
Skendzic and Keler, 2019	Fruit fly gut microbiome	Introductory UG	Biology
Cottone and Yoon, 2020	Leaf microbiomes	Introductory UG	Biology
Goller and Ott, 2020	Swab samples	Upper-level UG and Grad	Biotechnology
Parks et al., 2020	Winogradsky columns, urban microbiomes, kombucha	Introductory and upper-level UG	Microbiology, Microbial ecology
Riley et al., 2020	Gold-precipitating bacteria (*Delftia* spp.)	Introductory and upper-level UG	Interdisciplinary

Searching the available literature, this table lists articles that describe student microbiome research projects. The list is not likely to be exhaustive as other articles that fit the criteria may have been missed if the search terms were not present in the title, abstract, or key word fields. There are many other articles that describe excellent bioinformatics and genomics research projects but they are not included here if they did not explicitly mention a microbiome element to the research project. Abbreviations: UG = undergraduate, MS = masters, Grad = graduate, HS = high school, NS = not specified.

sequencing instruments became available to biologists in the mid-2000s (Goodwin et al., 2016; Slatko et al., 2018). This catapulted the depth of DNA sequence analysis from the few hundred reads that could be generated through clone libraries and Sanger sequencing, to hundreds of millions of reads generated by next-generation sequencing instruments. Together, culture-independent approaches and next-generation sequencing allow the study of complex, dynamic, microbial systems in greater detail than was previously possible. This work has led to the discovery of new phyla and has greatly increased our estimates of the diversity of bacteria, fungi and viruses in environments across the globe (Venter et al., 2004; Hug et al., 2016).

TEACHING QUANTITATIVE REASONING AND DATA SKILLS THROUGH MICROBIOME RESEARCH

Research experiences with microbiomes provide training for students in essential quantitative reasoning and data analysis skills that can be applied in the field of microbiology and many other STEM fields, as well as in non-STEM professions that are reliant on large and complex data sets (Chapman et al., 2006; Tan et al., 2009; Nelson and Campbell, 2011; Fenlon, 2017; Mulder et al., 2018; Attwood et al., 2019). There have been a number of recent reports that anticipate the needs of the research community and define the skills and experience students should have in order to enter research fields. A 2016 study surveyed more than 700 NSF principal investigators from the Biological Sciences Directorate (NSF-BIO) and asked where they saw unmet needs for analyzing "big data" for biological research (Barone et al., 2017). The top two categories of unmet data analysis needs were "training on integration of multiple data types" and "training on data management and metadata," and topping the list of major data types used by these PIs were DNA/RNA/protein sequence data. The Network for Integrating Bioinformatics into Life Sciences Education (NIBLSE) Core Competencies Working Group developed a set of 15 bioinformatics skills for undergraduate life sciences students and analyzed survey responses from 1260 biologists (Wilson Sayres et al., 2018). Their findings show the skills receiving the highest score ("extremely important" on a Likert-scale) include, "understand the role of computation and data mining in hypothesis-driven processes within the life sciences," "know statistical concepts used in bioinformatics," "know how to access genomic data," and "be able to use bioinformatics tools to analyze genomic data." The

study included a comparison of the relative importance of the bioinformatics skill and evidence that the skill was addressed in course syllabi, and found that statistics and metagenomics skills were among those with the greatest disparity between importance and actual representation in course syllabi (Wilson Sayres et al., 2018). In addition, NIBLSE surveyed biology faculty asking what they perceived as barriers to the integration of bioinformatics into undergraduate courses and categorized frequently cited barriers into six categories (Williams et al., 2019). Three categories: Faculty Issues, Student Issues, and Curriculum Issues, were the most cited by biology faculty, and within these categories, specific barriers included a lack of faculty expertise and a lack of faculty time, students' lack of background skills and students' lack of basic computing knowledge, as well as insufficient availability of bioinformatics lesson plans and the rapid rate at which bioinformatics material changed (Williams et al., 2019). These barriers are not easy to overcome, but might be reduced through the integration of microbiome research into courses (Cline and Prokop, 2018). Microbiome research allows students to work with large data sets, to learn statistical analyses, and to provide insight through data interpretation (references cited in **Table 1**). Several elements of the ASM curriculum guidelines, *Concepts and Statements* (American Society for Microbiology, 2012), can also be addressed through microbiome research projects. All of the ASM guidelines' *Scientific Thinking Skills*, which include, the "ability to apply the process of science," the "ability to use quantitative reasoning," and, the "ability to understand the relationship between science and society" can be addressed in microbiome research projects (**Figure 1**).

MICROBIOME PROJECTS IN UREs

Considering the training that benefits young scientists, and the skills that will be required in the biological research workforce, how can undergraduate microbiome research projects help to provide training and meet curriculum standards? Early work with students amplified genes of interest from total DNA isolated from environmental samples and sequenced clone libraries to identify previously undescribed bacteria (Boomer et al., 2002). This provided insight into the diversity of bacterial communities and employed bioinformatics tools such as BLAST and tree-building programs, but it did not generate the amount of data that easily led to quantitative and statistical analyses. The introduction of next-generation sequencing, in combination with culture-independent studies, however, released a flood of microbiome

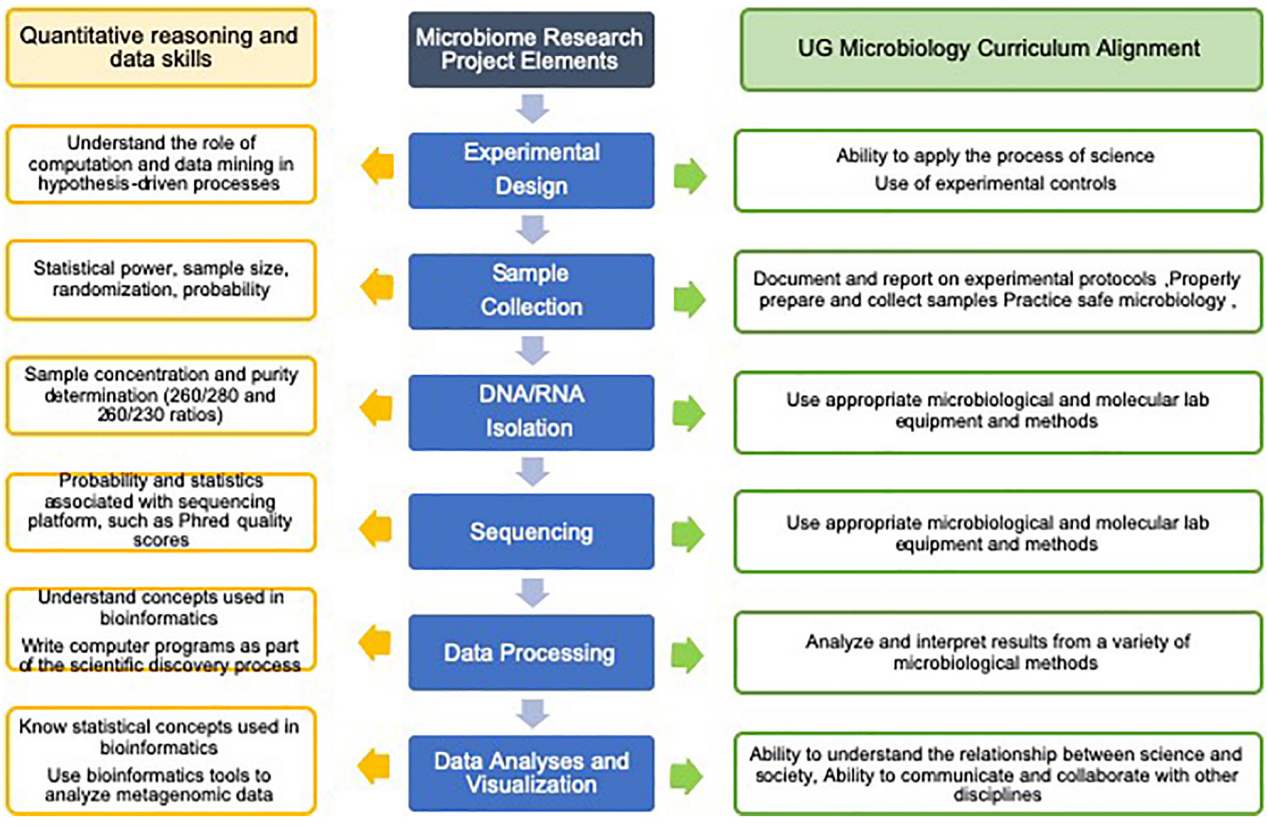

FIGURE 1 | Microbiome research projects can be designed to meet specific curriculum goals and to include quantitative reasoning and data skills. This figure illustrates how the basic elements of a standard microbiome research project can be aligned with the curriculum and specific data and analysis skills. Additional microbiology curriculum details and quantitative reasoning and data skills can be found in the references, American Society for Microbiology (2012) and Wilson Sayres et al. (2018).

sequence data and created an opportunity for students to contribute to the analysis. Using Global Ocean Sampling data sets, Hingamp et al. (2008) developed a parallel workflow to analyze reads from these data and students participated in "Annotathons" using bioinformatics tools to detect open reading frames and conserved domains, run BLAST searches and multiple sequence alignments, and construct phylogenetic trees (Rusch et al., 2007; Hingamp et al., 2008). Nearly 90% of the successfully classified sequences from the student Annotathons were bacteria, with an additional small percentage from Archaea and viruses. The student Annotathon process involves supervision and iteration to work toward a reliable sequence annotation, and is similar to models for student analysis of genomes and other biological data sets that had been developed earlier (Goodner et al., 2001; Hatfull et al., 2006; Slawson et al., 2006; Pico et al., 2008; Boyle, 2010; Ditty et al., 2010). Beyond sequence analysis, an ambitious ecological metagenomics course was developed for upper-level undergraduates and graduate students to contribute to on-going research of California sea lions and included surface marine water and kelp forest microbiome samples (Edwards et al., 2013). This interdisciplinary course took students through the process from DNA library preparation, next-generation sequencing, and data analysis, with student survey results demonstrating post-course acquisition of valuable wet-bench and data analysis skills. After initial "proof of concept" reports of investigating microbiomes with undergraduate students, a number of other publications followed that involved student studies of soil microbiomes, urban and built environment microbiomes, river microbiomes, plant and insect microbiomes, human microbiomes, and others (for details, see **Table 1**). While most CUREs involve learning practical laboratory skills as well as computational and bioinformatic skills, this is not always necessary. For example, Lentz et al. (2017) studied learning outcomes of a dry-lab approach using an open-access bioinformatic tool for analysis of human umbilical cord microbiomes. The positive learning outcomes included evaluating a hypothesis which is a skill usually associated with hands-on experimental design. The ability to engage students in data analysis research projects without a field or wet-bench component has been underscored by the current COVID-19 pandemic. In our experience, and from anecdotal reports of our colleagues, there are a number of undergraduate laboratory courses that have shifted focus to analyzing existing data sets with students online, and that have adapted approaches to meeting course learning objects that rely on remote, socially distanced, research experiences as a result of the constraints imposed by the pandemic. In parallel, we have observed an uptick in faculty demand for workshops and webinars that include emphasis on using data processing and analysis pipelines such as QIIME2 and mothur, as well as a desire to learn how to use more complex microbiome data analysis tools. These recent shifts underscore the flexibility of microbiome research and the potential to use microbiome projects as a scaffold for a range of learning objectives. While the social distancing measures necessitated by the pandemic will eventually be removed and allow faculty and students to resume in-person teaching, the impending budget crisis that many institutions will face is likely to require cost-cutting measures for years into the future. Shifting to microbiome data analysis with students, while not a perfect substitute for hands-on research experiences, could allow some courses to bridge a period of financial uncertainty by reducing the reagent and materials expenses that would be required for wet-bench labs.

ACCESSIBILITY – REACHING MORE STUDENTS WITH MICROBIOME PROJECTS

Cost and accessibility are factors in the equation when deciding whether or not to incorporate a microbiome URE into the curriculum. Microorganisms grow quickly, respond rapidly to environmental change, are highly diverse, and often are inexpensive to grow and maintain, making them ideal for classroom use. Using culture-independent approaches allows many microbiome projects to be carried out safely in BSL-1 level labs, to be run in high schools, and meaningful research projects to be designed and executed by students. This enables students to engage in the process of science and foster project ownership while producing data sets that allow microbiological questions to be addressed using quantitative analysis skills.

Although the cost of DNA sequencing continues to drop, many microbiome projects can still come with a hefty price tag. It is, however, possible to run exciting microbiome projects with students and generate excellent data with costs that fit modest budgets. Considering the arc of a microbiome project, costs for soil, water, or swab sample collection and DNA extraction range from $5 to $10 per sample, reagents for PCR amplification and quantifying DNA are $5 to $8 per sample, shipping samples to a sequencing facility are $35 to $50 for overnight shipments, the sequencing costs themselves can be $50 to $100 per sample depending on the platform used and number of reads per sample, and finally, data analysis can be free, for basic taxa tables, to more than $100 per sample for detailed analyses. From our experience of using microbiome UREs in laboratory courses, we spend $150 to $300 on reagents and sequencing costs for 2–4 independent samples in a section of 18 students per semester. Savings associated with removing older and less effective elements from the laboratory curriculum allowed the inclusion of the microbiome UREs at almost no additional cost over what had been budgeted for the lab without microbiome UREs. These costs are based on a laboratory that was equipped for standard work with DNA, including pipettors, gel electrophoresis equipment, spectrophotometer, centrifuge, and thermal cycler, and no additional major equipment purchases were required.

An important strategy in keeping costs down is to have students work in small groups of three to six students, and to pool multiple DNA samples into a single sample for sequencing. In the analysis of complex and heterogenous environments, such as soil, it is recommended to collect a number of independent samples from a specified plot in order to accurately represent the microbial community (Knight et al., 2018). Having multiple

student groups prepare independent DNA samples from the same site, and combining these samples into a composite sample for sequencing, both reduces costs and provides a better picture of the microbial community present at the sample site. For example, a lab of 18 students working in six groups of three can compare communities from two different sites, or compare an experimental treatment to a control, with each group preparing two DNA samples (for ∼$120) and sequencing only two composite samples (for ∼$120) is a savings of ∼$600 compared to the cost of sequencing each isolated DNA sample.

It is, however, an unfortunate reality that even a few hundred dollars can be too costly for some budgets, and if essential equipment is lacking, student microbiome projects can be pushed out of the reach of many classrooms. While the desired solution is that science education receives the funding that is required to train and prepare students in STEM disciplines, it is a fact that many public institutions, and institutions in underserved communities, simply are not funded adequately and they must turn to cost-cutting compromises. In surveys we have administered to faculty, the cost of microbiome projects is among the most significant barriers to their implementation as UREs. Working with our colleagues at REMNet (an NSF-funded RCN-UBE), it has been part of our mission to facilitate student microbiome research projects and to find creative ways to make projects accessible to as wide a range of classrooms as possible. In addition to the cost saving practices described above, REMNet has encouraged the introduction of research elements into the classroom through CUREs. Through "dual use" design, faculty can bring down the costs of CURE microbiome projects by aligning them with the goals of their own laboratory research, or through collaborations with research projects lead by investigators at their institution or nearby institutions. The coordination of CURE student research with faculty research projects can result in students' projects contributing preliminary data for competitive grants aimed at supporting additional research. Professional development and training can also be significant costs for faculty that prevent the incorporation of microbiome projects into the curriculum. The REMNet community has been able to provide training and support for faculty through workshops, online videos, and a collection of protocols that have been developed for student microbiome projects. Finally, the overwhelming amount of data generated by many microbiome projects creates an opportunity for many downstream data analysis projects. For labs or courses without a wet bench component, or for those labs without access to essential equipment, it is possible to collaborate with other investigators who can provide sequence data to be analyzed by students. These shared data can serve as the basis of novel and demanding UREs that center on data analysis and data visualization. Through efforts such as those mentioned here, and other creative approaches, it is possible to make undergraduate microbiome research projects accessible.

Reports, such as Vision and Change, call for engaging students in the process of science and argue that greater engagement results in positive outcomes in student success, learning, problem solving, and an appreciation for research (Anderson et al., 2011; Vision and Change in Undergraduate Biology Education, 2011; Merkel, 2012). The default for undergraduate research is based on a faculty-mentored apprenticeship model, where students work on a project over a semester or longer. Students who engage in undergraduate research in this way show improved academic outcomes and greater levels of graduate school admissions (Cooper et al., 2019b; Nerio et al., 2019). A major limitation in the apprenticeship model, however, is that it is constrained by the number of faculty who can take on students in their lab. In many institutions, particularly community colleges and public colleges and universities, the potential demand for research experiences can exceed apprenticeship capacity by 10-fold or more (Parks et al., 2020). Accordingly, solutions have been sought that broaden student research training, such as intensive summer research programs and CUREs. While many CUREs have been developed recently, there is a track record of success, and some programs, such as the Superlab at Haverford College, have been training students using a CURE model for more than 50 years (Owen et al., 1991; Alkaher and Dolan, 2014).

Determining which mode of URE is the best fit for a microbiome research project requires coordination of the learning goals for students with the resources of time, funding, faculty expertise, and any parallel research objectives. A best practice in achieving this coordination is based on an incremental approach that begins with piloting a project on a small scale before moving on to incorporating a project into a large course, or courses with multiple sections and instructors. Small pilot projects provide an opportunity to identify potential problems in scaling-up and can produce initial data that are helpful in convincing colleagues and administrators that a larger microbiome project implementation is feasible. Traditional faculty-mentored research projects, small capstone courses, and summer research experiences often are formats that are well suited for the piloting phase. These formats can be stepping stones to larger and more ambitious microbiome CURE projects. As an example of this, the Authentic Research Experiences in Microbiology (AREM) program began with three undergraduate students working in a faculty lab as part of an independent research course studying urban microbiomes from city playgrounds, subway stations, and soil from local parks (Muth and McEntee, 2014). After adapting protocols for use with student groups and determining how the experiments would map onto the course schedule, the microbiome project was incorporated as a multi-week module in a single undergraduate microbiology lab section of 18 students (Biology majors in their 2nd or 3rd year of study). After two semesters in a single lab section, a second laboratory section was converted to include the AREM microbiome research module. At the same time a set of basic assessment tools were used that allowed a comparison of student learning and attitudes with microbiology lab sections being run at the same time using the traditional format without the AREM microbiome research module. The initial results showed that there was greater student engagement and excitement in the sections that included the microbiome research component, and gradually, the microbiome research module was incorporated into all ten laboratory sections over the next two semesters. Even with this step-wise approach, challenges

remain, the most significant being the need to train additional instructors teaching the AREM module.

In addition to piloting microbiome research projects, it is helpful to start with well-defined projects with a narrow scope. Isolating and studying microbiomes from diverse natural, urban, and human environments has an inherent exploratory element that appeals to students, however, the study of microcosms in laboratory settings, such as Winogradsky columns, allows students to investigate how controlled experimental variables influence microbiomes (Rundell et al., 2014; Parks, 2015; Parks et al., 2020). Laboratory maintained microcosms are often better suited to comparative studies with proper controls and replication, and can be run in winter when collecting outdoor samples may not be an option. Akin to Winogradsky columns, in a modified AREM module, students use soil samples collected from the campus to test a hypothesis related to the competitive exclusion of potential pathogens. Each student group prepares three soil conditions, (a) one tube of 10 g of soil, (b) one tube of 10 g of soil spiked with a laboratory strains of *E. coli* and *S. epidermidis*, and (c) one tube of 10 g of autoclaved soil spiked with a laboratory strains of *E. coli* and *S. epidermidis*. Total DNA is extracted from 250 mg of soil from each tube on day one and again 3 weeks later. The multiple student groups provide the replication for this experiment. In a simple and inexpensive design such as this, students can develop and test a hypothesis, quantify the diversity and relative abundance of bacteria in a local soil sample, measure colonization resistance, see the limitations posed by the presence of relic DNA, as well as other questions. Controlled variables sometimes exist in the environment, as was the case for students studying the effect of soil temperature on bacterial communities isolated from above the Centralia, PA mine fire (Tobin and Shade, 2018), and fertilizer additions to agricultural soils (Costas et al., 2017). Selecting a question of interest, formulating a hypothesis, and developing an experimental design to test the hypothesis are important features of the process of science (Hoskins et al., 2011; Goldey et al., 2012; Smith et al., 2013). Including students in these steps can add to the value of a microbiome URE and can better target learning objectives than survey projects that study microbiomes from the environment without a guiding hypothesis.

There are many excellent examples of microbiology UREs that broaden participation and reach a demographic of students who might not otherwise engage in research (Eagan et al., 2013; Bangera and Brownell, 2014; Schinske et al., 2017). A number of large projects involve students in microbiology research on a national scale, including, Tiny Earth (Handelsman et al., 2018; Basalla et al., 2020), Small World Initiative (Hernandez et al., 2015; Davis et al., 2016), Prevalence of Antibiotic Resistance in the Environment (PARE) (Genné-Bacon and Bascom-Slack, 2019), Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) project (Jordan et al., 2014), the Microbial Genome Annotation Network (MGAN), and AREM (Muth and McEntee, 2014). Having a diversity of options is useful because microbiology instruction occurs in many different contexts, with wide variations in faculty expertise, research interests, time availability, resources and infrastructure, student preparation, and curriculum requirements. While no single solution will suffice, microbiome research projects can be versatile and exciting, and are being used extensively in UREs, citizen science projects, and K-12 classes. Varying formats of microbiome research can be tailored to specific research and curricular goals. These include project-based, upper-level laboratory courses and intensive summer research programs where students self-select for participation and may have a number of pre-requisites to meet. Implementations such as these tend to be small sections with multiple experienced instructors who work closely with students to help them master complex sample preparation and sequencing protocols (Edwards et al., 2013; Rundell et al., 2014; Muterspaw et al., 2015). In a smaller class with expert support and sufficient access to computers, it is also feasible to carry out sequence filtering and processing using command line interface and pipelines such as QIIME2 (Bolyen et al., 2019). These are fantastic experiences for students and, with the appropriate design, they can generate publication quality data.

However, not every institution or department is able to run such a course, and even when possible, the smaller class size prevents many students from having an opportunity to participate before they graduate. Our experience with undergraduate microbiome research began at the City University of New York, the country's largest urban university, and an environment that, because of its size and modest means, is only able to reach a small fraction of students with project-based, upper-level laboratory courses. We developed AREM as a flexible, modular microbiome research approach that faculty could integrate into existing courses and across multiple sections, without significant expense or logistical hurdles (Muth and McEntee, 2014). In most implementations, the modular AREM microbiome projects have a focus on microbiology content and less so on sequencing, data processing, and bioinformatics elements. It is important to align a microbiome research project with curricular goals, and in an introductory microbiology course it may be a better fit to emphasize microbial diversity, phylogeny, growth, competition, metabolism, and environmental influences, rather than devoting several sessions to the intricacies of data processing and advanced data analyses. In 16S amplicon sequencing-based projects, it is standard for most commercial and institutional sequencing facilities to provide a taxonomy table with relative abundances in a spreadsheet format, and this is often more than enough data for most courses, and precludes the need for student or faculty expertise using data processing pipelines. Using the AREM design, modular microbiome projects based on 16S sequence data sets have been used with high school students, at community colleges, at primarily undergraduate institutions, and at large universities. The core elements of experimental design, samples collection, DNA isolation, sequencing, and basic data analysis run through most implementations of modular microbiome projects and are easily adapted to fit specific course requirements, research goals, and learning objectives (**Figure 1**). Several experienced practitioners have worked together to establish the REMNet, and this network provides expert support and training resources to faculty working with students on

microbiome research projects. The REMNet community expects to grow and develop, and to provide support for a wider range of amplicon sequencing, metagenomic, RNAseq, and other data types.

Looking beyond direct benefits to the scientific research community, a successful microbiome project should help students to develop technical expertise, and more generally, should help to develop their critical thinking skills and further their scientific understanding and ability to communicate their ideas. This is essential to the changing needs of the workforce that increasingly requires strengths in critical thinking, problem solving, and the ability to collaborate with colleagues. An important aspect of microbiome projects is engaging students by addressing questions without a predetermined outcome. A focus on unknown microbial communities excites and resonates with students because it involves the environments where they live, study, and work. The questions they ask, the data they collect, and the interpretations of those data are relevant to them and they are more likely to have a sense of ownership of the project. This engagement and project ownership can translate into a certain degree of pride and social responsibility, as students often talk about how they may have changed their own opinions, and the opinions of friends and family, in regards to the role of microorganisms in the environment and the connection to environmental and human health.

SUMMARY

Microbiome research projects are accessible to undergraduate students. From the development of research questions related to microbiomes, to DNA sequence analysis and data interpretation, students find themselves integrating information from the fields of genetics, ecology, statistics, epidemiology and health sciences. The involvement of interdisciplinary work underscores in the important collaborative nature of research and the need to exchange ideas and perspectives.

Microbiome studies may begin with unrelated research questions and different sampling sources, but the sequence output from most projects can be analyzed with a shared set of tools and allows for training in quantitative and data analysis skills. This underscores the versatility of microbiome studies in providing students with an opportunity to practice and learn from the exploration of large data sets using bioinformatics, data analysis, and data visualization tools. Microbiome research projects can be tied directly to questions on related to biodiversity and ecology, as well as topics that have social justice components such as climate change, food security, and human health. The relevance of these research areas to issues students encounter in the news and to issues discussed in public fora is attractive because it ties students' academic studies directly to the real-world.

AUTHOR CONTRIBUTIONS

TM and AC contributed equally to the preparation of the text for this manuscript. TM assembled the tables and figures for the manuscript. All the authors contributed to the article and approved the submitted version.

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REFERENCES

Abid, M. B. (2019). Could the menagerie of the gut microbiome really cure cancer? Hope or hype. *J. Immuno Therapy Cancer* 7, 1–7. doi: 10.1186/s40425-019-0561-9

Alessi, A. M., Redeker, K. R., and Chong, J. P. J. (2018). A practical introduction to microbial molecular ecology through the use of isolation chips. *Ecol. Evol.* 8, 12286–12298. doi: org/10.1002/ece3.4748

Alkaher, I., and Dolan, E. L. (2014). "Integrating research into undergraduate courses: current practices and future directions," in *Research in Science Education*, eds D. Sunal, C. Sunal, E. Wright, C. Mason, and D. Zollman (Charlotte, NC: Information Age Publishing).

Almeida, A., Mitchell, A. L., Boland, M., Forster, S. C., Gloor, G. B., Tarkowska, A., et al. (2019). A new genomic blueprint of the human gut microbiota. *Nature* 568, 499–504. doi: 10.1038/s41586-019-0965-1

American Society for Microbiology (2012). *Recommended Curriculum Guidelines for Undergraduate Microbiology Education*. Washington DC: American Society for Microbiology.

American Society for Microbiology (2019). *ASM Guidelines for Biosafety in Teaching Laboratories*. Washington DC: American Society for Microbiology.

Anderson, W. A., Banerjee, U., Drennan, C. L., Elgin, S. C. R., Epstein, I. R., Handelsman, J., et al. (2011). Changing the culture of science education at research universities. *Science* 331, 152–153. doi: 10.1126/science.1198280

Attwood, T. K., Blackford, S., Brazas, M. D., Davies, A., and Schneider, M. V. (2019). A global perspective on evolving bioinformatics and data science training needs. *Brief. Bioinform.* 20, 398–404. doi: 10.1093/bib/bbx100

Auchincloss, L. C., Laursen, S. L., Branchaw, J. L., Eagan, K., Graham, M., Hanauer, D. I., et al. (2014). Assessment of course-based undergraduate research experiences: ameeting report. *CBE Life Sci. Educ.* 13, 29–40. doi: 10.1187/cbe.14-01-0004

Bangera, G., and Brownell, S. E. (2014). Course-based undergraduate research experiences can make scientific research more inclusive. *Sci. Educ.* 13, 602–606. doi: 10.1187/cbe.14-06-0099

Barea, J. M. (2015). Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. *J. Soil Sci. Plant Nutr.* 15, 261–282.

Barone, L., Williams, J., and Micklos, D. (2017). Unmet needs for analyzing biological big data: a survey of 704 NSF principal investigators. *PLoS Comput. Biol.* 13:5755. doi: 10.1371/journal.pcbi.1005755

Basalla, J., Harris, R., Burgess, E., Zeedyk, N., and Wildschutte, H. (2020). Expanding Tiny Earth to genomics: a bioinformatics approach for an undergraduate class to characterize antagonistic strains. *FEMS Microbiol. Lett.* 367:29. doi: 10.1093/femsle/fnaa018

Bik, E. M. (2016). The hoops, hopes, and hypes of human microbiome research. *Yale J. Biol. Med.* 89, 363–373. doi: 27698620

Blaser, M. J., Cardon, Z. G., Cho, M. K., Dangl, J. L., Donohue, T. J., Green, J. L., et al. (2016). Toward a predictive understanding of earth's microbiomes to address 21st century challenges. *mBio* 7, 1–16. doi: 10.1128/mBio.00714-16

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* 37, 852–857. doi: 10.1038/s41587-019-0209-9

Boomer, S. M., Lodge, D. P., Dutton, B. E., and Pierson, B. (2002). Molecular characterization of novel red green nonsulfur bacteria from five distinct hot spring communities in Yellowstone National Park. *Appl. Environ. Microbiol.* 68, 346–355. doi: 10.1128/AEM.68.1.346-355.2002

Boyle, M. D. (2010). Shovel-ready sequences as a stimulus for the next generation of life scientists. *J. Microbiol. Biol. Educ.* 11, 38–41. doi: 10.1128/jmbe.v11.i1.133

Brown, J. M., and Hazen, S. L. (2017). Targeting of microbe-derived metabolites to improve human health: the next frontier for drug discovery. *J. Biol. Chem.* 292, 8560–8568. doi: 10.1074/jbc.R116.765388

Buonaccorsi, V. P., Boyle, M. D., Grove, D., Praul, C., Sakk, E., Stuart, A., et al. (2011). GCAT-SEEKquence: genome consortium for active teaching of undergraduates through increased faculty access to next-generation sequencing data. *CBE Life Sci. Educ.* 10, 342–345. doi: 10.1187/cbe.11-08-0065

Chapman, B. S., Christmann, J. L., and Thatcher, E. F. (2006). Bioinformatics for undergraduates: steps toward a quantitative bioscience curriculum. *Biochem. Mol. Biol. Educ.* 34, 180–186. doi: 10.1002/bmb.2006.49403403180

Cline, S. G., and Prokop, J. W. (2018). Framework, barriers, and proposed solutions for engaging students in bioinformatics research. *Proc. Int. Conf. Bioinform. Comput. Biol.* 1, 42–46.

Coil, D. A., Ettinger, C. L., and Eisen, J. A. (2017). Gut check: the evolution of an educational board game. *PLoS Biol.* 15:84. doi: 10.1371/journal.pbio.2001984

Cooper, K. M., Blattman, J. N., Hendrix, T., and Brownell, S. E. (2019a). The impact of broadly relevant novel discoveries on student project ownership in a traditional lab course turned CURE. *CBE Life Sci. Educ.* 18, 1–14. doi: 10.1187/cbe.19-06-0113

Cooper, K. M., Gin, L. E., Akeeh, B., Clark, C. E., Hunter, J. S., Roderick, T. B., et al. (2019b). Factors that predict life sciences student persistence in undergraduate research experiences. *PLoS One* 14:186. doi: 10.1371/journal.pone.0220186

Costas, A. G., Ragen, D. L., and Peters, J. W. (2017). Searching for nitrogen-fixing microorganisms: an original, relevant, and successful early research experience. *Am. Biol. Teach.* 79, 191–197. doi: 10.1525/abt.2017.79.3.191

Cottone, A. M., and Yoon, S. (2020). Improving the design of undergraduate biology courses toward the goal of retention: the case of real-world inquiry and active learning through metagenomics. *J. Microbiol. Biol. Educ.* 21, 1–10. doi: 10.1128/JMBE.V21I1.1965

Davis, E., Sloan, T., Aurelius, K., Barbour, A., Bodey, E., Clark, B., et al. (2016). Antibiotic discovery throughout the small world initiative: a molecular strategy to identify biosynthetic gene clusters involved in antagonistic activity. *Microbiol. Open* 6:e00435. doi: 10.1002/mbo3.435

Ditty, J. L., Kvaal, C. A., Goodner, B., Freyermuth, S. K., Bailey, C., Britton, R. A., et al. (2010). Incorporating genomics and bioinformatics across the life sciences curriculum. *PLoS Biol.* 8:448. doi: 10.1371/journal.pbio.1000448

Docherty, K. M., Borton, H. M., Espinosa, N., Gebhardt, M., Gil-Loaiza, J., Gutknecht, J. L. M., et al. (2015). Key edaphic properties largely explain temporal and geographic variation in soil microbial communities across four biomes. *PLoS One* 10:352. doi: 10.1371/journal.pone.0135352

Donato, J. J., Klimstra, M. A., Byrnes, J. R., White, R. J., Marsh, T. C., et al. (2012). The introduction of metagenomics into an undergraduate biochemistry laboratory course yielded a predicted reductase that decreases triclosan susceptibility in *Escherichia coli*. *DNA Cell Biol.* 31, 968–973. doi: 10.1089/dna.2011.1512

Eagan, M. K. Jr., Hurtado, S., Chang, M. J., Garcia, G. A., Herrera, F. A., Garibay, J. C., et al. (2013). Making a difference in science education: the impact of undergraduate research programs. *Am. Educ. Res. J.* 50, 683–713. doi: 10.3102/0002831213482038

Edwards, R. A., Haggerty, J. M., Cassman, N., Busch, J. C., Aguinaldo, K., Chinta, S., et al. (2013). Microbes, metagenomes and marine mammals: enabling the next generation of scientist to enter the genomic era. *BMC Genomics* 14:600. doi: 10.1186/1471-2164-14-600

Escobar-Zepeda, A., De León, A. V., and Sanchez-Flores, A. (2015). The road to metagenomics: from microbiology to DNA sequencing technologies and bioinformatics. *Front. Genet.* 6:348. doi: 10.3389/fgene.2015.00348

Estes, A. M. (2015). Modeling the dynamic digestive system microbiome. *J. Microbiol. Biol. Educ.* 16, 271–273. doi: 10.1128/jmbe.v16i2.908

Falkowski, P. G., Fenchel, T., and Delong, E. F. (2008). The microbial engines that drive earth's biogeochemical cycles. *Science* 320, 1034–1039. doi: 10.1126/science.1153213

Fenlon, M. (2017). Investing in America's data science and analytics talent. *PwC Bus. High. Educ. Forum* 1, 1–24. doi: 10.1002/9781119083856.ch1

Finbow, A. (2019). How microbiomes could save the planet. *Sci. Am.* 321, 1–3. doi: 10.2307/j.ctt20mvdqx.4

Frank, D. N., and Pace, N. R. (2008). Gastrointestinal microbiology enters the metagenomics era. *Curr. Opin. Gastroenterol.* 24, 4–10. doi: 10.1097/MOG.0b013e3282f2b0e8

Freeman, S., Okoroafor, N. O., Gast, C. M., Koval, M., Nowowiejski, D., O'Connor, E., et al. (2016). Crowdsourced data indicate widespread multidrug resistance in skin flora of healthy young adults. *J. Microbiol. Biol. Educ.* 17, 172–182. doi: 10.1128/jmbe.v17i1.1008

Genné-Bacon, E. A., and Bascom-Slack, C. A. (2019). The PARE project: a short course-based research project for national surveillance of antibiotic-resistant microbes in environmental samples. *J. Microbiol. Biol. Educ.* 19, 1–7. doi: 10.1128/jmbe.v19i3.1603

Gibbens, B. B., Scott, C. L., Hoff, C. D., and Schottel, J. L. (2015). Exploring metagenomics in the laboratory of an introductory biology course. *J. Microbiol. Biol. Educ.* 16, 34–40. doi: 10.1128/jmbe.v16i1.780

Goldey, E. S., Abercrombie, C. L., Ivy, T. M., Kusher, D. I., Moeller, J. F., Rayner, D. A., et al. (2012). Biological inquiry: a new course and assessment plan in response to the call to transform undergraduate biology. *CBE Life Sci. Educ.* 11, 353–363. doi: 10.1187/cbe.11-02-0017

Goller, C. C., and Ott, L. E. (2020). Evolution of an 8-week upper-division metagenomics course: diagramming a learning path from observational to quantitative microbiome analysis. *Biochem. Mol. Biol. Educ.* 48, 391–403. doi: 10.1002/bmb.21349

Goodner, B., Hinkle, G., Gattung, S., Miller, N., Blanchard, M., Qurollo, B., et al. (2001). Genome sequence of the plant pathogen and biotechnology agent *Agrobacterium tumefaciens* C58. *Science* 294, 2323–2328. doi: 10.1126/science.1066803

Goodwin, S., McPherson, J. D., and McCombie, W. R. (2016). Coming of age: ten years of next-generation sequencing technologies. *Nat. Rev. Genet.* 17, 333–351. doi: 10.1038/nrg.2016.49

Gutell, R. R., Larsen, N., and Woese, C. R. (1994). Lessons from an evolving rRNA: 16S and 23S rRNA structures from a comparative perspective. *Microbiol. Rev.* 58, 10–26. doi: 10.1128/mmbr.58.1.10-26.1994

Hanauer, D. I., and Dolan, E. L. (2014). The project ownership survey: measuring differences in scientific inquiry experiences. *CBE Life Sci. Educ.* 13, 149–158. doi: 10.1187/cbe.13-06-0123

Handelsman, J. (2004). Metagenomics: application of genomics to uncultured microorganisms. *Microbiol. Mol. Biol. Rev.* 68, 669–685. doi: 10.1128/MMBR.68.4.669-685.2004

Handelsman, J., Tsang, T., Bascom-Slack, C., and Broderick, N. (2018). *Tiny Earth – A Research Guide to Student Sourcing Antibiotic Discovery*. San Jose, CA: eBay Inc. doi: 10.1128/mmbr.68.4.669-685.2004

Hartman, M. R., Harrington, K. T., Etson, C. M., Fierman, M. B., Slonim, D. K., Walt, D. R., et al. (2016). Personal microbiomes and next-generation sequencing for laboratory-based education. *FEMS Microbiol. Lett.* 363, 1–4. doi: 10.1093/femsle/fnw266

Harvey, A. L., Edrada-Ebel, R., and Quinn, R. J. (2015). The re-emergence of natural products for drug discovery in the genomics era. *Nat. Rev. Drug Discov.* 14, 111–129. doi: 10.1038/nrd4510

Hatfull, G. F., Pedulla, M. L., Jacobs-Sera, D., Cichon, P. M., Foley, A., Ford, M. E., et al. (2006). Exploring the mycobacteriophage metaproteome: Phage genomics as an educational platform. *PLoS Genet.* 2:92. doi: 10.1371/journal.pgen.0020092

Hernandez, S., Tsang, T., Bascom-Slack, C., and Handelsman, J. (2015). *Small World Initiative. 4th Edition. Editors Nichole Broderick and Erika Kurt*. Acton MA: Small World Initiative Press.

Hingamp, P., Brochier, C., Talla, E., Gautheret, D., Thieffry, D., Herrmann, C., et al. (2008). Metagenome annotation using a distributed grid of undergraduate students. *PLoS Biol.* 6:296. doi: 10.1371/journal.pbio.0060296

Hoskins, S. G., Lopatto, D., and Stevens, L. M. (2011). The C.R.E.A.T.E. approach to primary literature shifts undergraduates' self-assessed ability to read and analyze journal articles, attitudes about science, and epistemological beliefs. *CBE Life Sci. Educ.* 10, 368–378. doi: 10.1187/cbe.11-03-0027

Hotaling, S., Slabach, B. L., and Weisrock, D. W. (2018). Next-generation teaching: a template for bringing genomic and bioinformatic tools into the classroom. *J. Biol. Educ.* 52, 301–313. doi: 10.1080/00219266.2017.1357650

Hug, L. A., Baker, B. J., Anantharaman, K., Brown, C. T., Probst, A. J., Castelle, C. J., et al. (2016). A new view of the tree of life. *Nat. Microbiol.* 1, 1–6. doi: 10.1038/nmicrobiol.2016.48

Jez, J. M., Lee, S. G., and Sherp, A. M. (2016). The next green movement: plant biology for the environment and sustainability. *Science* 353, 1241–1244. doi: 10.1126/science.aag1698

Jordan, T. C., Burnett, S. H., Carson, S., Caruso, S. M., Clase, K., DeJong, R. J., et al. (2014). A broadly implementable research course in phage discovery and genomics for first-year undergraduate students. *mBio* 5, 1–8. doi: 10.1128/mBio.01051-13

Jurkowski, A., Reid, A. H., and Labov, J. B. (2007). Metagenomics: a call for bringing a new science into the classroom (while it's still new). *CBE Life Sci. Educ.* 6, 260–265. doi: 10.1187/cbe.07-09-0075

King, G. M. (2014). Urban microbiomes and urban ecology: how do microbes in the built environment affect human sustainability in cities? *J. Microbiol.* 52, 721–728. doi: 10.1007/s12275-014-4364-x

Kloser, M. J., Brownell, S. E., Chiariello, N. R., and Fukami, T. (2011). Integrating teaching and research in undergraduate biology laboratory education. *PLoS Biol.* 9:1001174. doi: 10.1371/journal.pbio.1001174

Knight, R., Vrbanac, A., Taylor, B., Aksenov, A., Callewaert, C., Debelius, J., et al. (2018). Best practices for analyzing microbiomes. *Nat. Rev. Microbiol.* 16, 410–422. doi: 10.1038/s41579-018-0029-9

Krüger, A., Schäfers, C., Schröder, C., and Antranikian, G. (2018). Towards a sustainable biobased industry – Highlighting the impact of extremophiles. *New Biotechnology* 40, 144–153. doi: 10.1016/j.nbt.2017.05.002

Lentz, T. B., Ott, L. E., Robertson, S. D., Windsor, S. C., Kelley, J. B., Wollenberg, M. S., et al. (2017). Unique down to our microbes—assessment of an inquiry-based metagenomics activity. *J. Microbiol. Biol. Educ.* 18, 1–8. doi: 10.1128/jmbe.v18i2.1284

Locey, K. J., and Lennon, J. T. (2016). Scaling laws predict global microbial diversity. *Proc. Natl. Acad. Sci. U.S.A.* 113, 5970–5975. doi: 10.1073/pnas.1521291113

Lopatto, D. (2010). Undergraduate research as a high-impact student experience. *Peer Rev.* 12, 1–1.

Merkel, S. (2012). The development of curricular guidelines for introductory microbiology that focus on understanding. *J. Microbiol. Biol. Educ.* 13, 32–38. doi: 10.1128/jmbe.v13i1.363

Mulder, N., Schwartz, R., Brazas, M. D., Brooksbank, C., Gaeta, B., Morgan, S. L., et al. (2018). The development and application of bioinformatics core competencies to improve bioinformatics training and education. *PLoS Comput. Biol.* 14:5772. doi: 10.1371/journal.pcbi.1005772

Muterspaw, K., Urner, T., Lewis, R., Babic, I., Srinath, D., Peck, C., et al. (2015). Multidisciplinary research and education with open tools: metagenomic analysis of 16S rRNA using Arduino, Android, Mothur and XSEDE. *ACM Int. Conf. Proc. Ser.* 22, 1–8. doi: 10.1145/2792745.2792767

Muth, T. M., and McEntee, C. (2014). Undergraduate Urban metagenomics research module. *J. Microbiol. Biol. Educ.* 15, 38–40. doi: 10.1128/jmbe.v15i1.645

National Research Council (2007). *New Science of Metagenomics*. Washington, DC: The National Academies Press.

Nelson, J., and Campbell, C. (2011). Evidence-informed practice in education: meanings and applications. *Educ. Res.* 59, 127–135. doi: 10.1080/00131881.2017.1314115

Nerio, R., Webber, A., MacLachlan, E., Lopatto, D., and Caplan, A. J. (2019). One-year research experience for associate's degree students impacts graduation, stem retention, and transfer patterns. *CBE Life Sci. Educ.* 18:ar25. doi: 10.1187/cbe.19-02-0042

Nielsen, P. H., Saunders, A. M., Hansen, A. A., Larsen, P., and Nielsen, J. L. (2012). Microbial communities involved in enhanced biological phosphorus removal from wastewater-a model system in environmental biotechnology. *Curr. Opin. Biotechnol.* 23, 452–459. doi: 10.1016/j.copbio.2011.11.027

Owen, J. A., Matacic, S. S., Iwaseczko, T., and Dalesandro, M. R. (1991). Design of an undergraduate laboratory to generate fluorescein-specific monoclonal antibodies. *J. Biotechnol. Educ.* 2, 108–112.

Parks, S. (2015). Microbial life in a winogradsky column: from lab course to diverse research experience. *J. Microbiol. Biol. Educ.* 16, 82–84. doi: 10.1128/jmbe.v16i1.847

Parks, S., Joyner, J. L., and Nusnbaum, M. (2020). Reaching a large urban undergraduate population through microbial ecology course-based research experiences. *J. Microbiol. Biol. Educ.* 21, 1–112. doi: 10.1128/JMBE.V21I1.2047

Pico, A. R., Kelder, T., Van Iersel, M. P., Hanspers, K., Conklin, B. R., Evelo, C., et al. (2008). WikiPathways: Pathway Editing for the People. *PLoS Biol.* 6:184. doi: 10.1371/journal.pbio.0060184

Podolsky, I. A., Seppälä, S., Lankiewicz, T. S., Brown, J. L., Swift, C. L., O'Malley, M. A., et al. (2019). Harnessing nature's anaerobes for biotechnology and bioprocessing. *Ann. Rev. Chem. Biomol. Eng.* 10, 105–128. doi: 10.1146/annurev-chembioeng-060718-030340

Riesenfeld, C. S., Schloss, P. D., and Handelsman, J. (2004). Metagenomics: genomic analysis of microbial communities. *Ann. Rev. Genet.* 38, 525–552. doi: 10.1146/annurev.genet.38.072902.091216

Riley, N. G., Goller, C. C., Leggett, Z. H., Lewis, D. M., Ciccone, K., Dunn, R. R., et al. (2020). Catalyzing rapid discovery of gold-precipitating bacterial lineages with university students. *PeerJ* 8:e8925. doi: 10.7717/peerj.8925

Rios-Velazquez, C., Williamson, L. L., Cloud-Hansen, K. A., Allen, H. K., McMahon, M. D., Sabree, Z. L., et al. (2011). Summer workshop in metagenomics: one week plus eight students equals gigabases of cloned DNA. *J. Microbiol. Biol. Educ.* 12, 120–126. doi: 10.1128/jmbe.v12i2.177

Robertson-Albertyn, S. (2016). Microbe motels: an interactive method to introduce the human microbiome. *J. Microbiol. Biol. Educ.* 17, 282–283. doi: 10.1128/jmbe.v17i2.966

Rundell, E. A., Banta, L. M., Ward, D. V., Watts, C. D., Birren, B., Esteban, D. J., et al. (2014). 16S rRNA Gene Survey of Microbial Communities in Winogradsky columns. *PLoS ONE* 9:1–14. doi: 10.1371/journal.pone.0104134

Rusch, D. B., Halpern, A. L., Sutton, G., Heidelberg, K. B., Williamson, S., Yooseph, S., et al. (2007). The sorcerer II global ocean sampling expedition: northwest atlantic through eastern tropical Pacific. *PLoS Biol.* 5:e77. doi: 10.1371/journal.pbio.0050077

Sanders, E. R., Moberg-Parker, J., Hirsch, A. M., Lee, P. Y., Shapiro, C., Toma, S., et al. (2016). Transforming laboratory education in the life sciences: a scalable framework for designing authentic undergraduate research experience-based courses benefits both students and faculty. *Microbe* 11, 69–74. doi: 10.1128/microbe.11.69.1

Schinske, J. N., Balke, V. L., Bangera, M. G., Bonney, K. M., Brownell, S. E., Carter, R. S., et al. (2017). Broadening participation in biology education research: engaging community college students and faculty. *CBE Life Sci. Educ.* 16, 1–11. doi: 10.1187/cbe.16-10-0289

Schmulson, M., and Bashashati, M. (2018). Fecal microbiota transfer for bowel disorders: efficacy or hype? *Curr. Opin. Pharmacol.* 43, 72–80. doi: 10.1016/j.coph.2018.08.012

Scott Weber, K., Bridgewater, L. C., Jensen, J. L., Breakwell, D. P., Nielsen, B. L., Johnson, S. M., et al. (2018). Personal microbiome analysis improves student engagement and interest in immunology, molecular biology, and genomics undergraduate courses. *PLoS One* 13:e0193696. doi: 10.1371/journal.pone.0193696

Skendzic, E., and Keler, C. (2019). Fruit flies & the gut microbiome: redesign-your-bacteria lab exercise. *Am. Biol. Teacher* 81, 47–51. doi: 10.1525/abt.2019.81.1.47

Slatko, B. E., Gardner, A. F., and Ausubel, F. M. (2018). Overview of next-generation sequencing technologies. *Curr. Protoc. Mol. Biol.* 122, 1–2. doi: 10.1002/cpmb.59

Slawson, E. E., Shaffer, C. D., Malone, C. D., Leung, W., Kellmann, E., Shevchek, R. B., et al. (2006). Comparison of dot chromosome sequences from D.

melanogaster and D. virilis reveals an enrichment of DNA transposon sequences in heterochromatic domains. *Genome Biol.* 7, 1–18. doi: 10.1186/gb-2006-7-2-r15

Smith, J. J., Cheruvelil, K. S., and Auvenshine, S. (2013). Assessment of student learning associated with tree thinking in an undergraduate introductory organismal biology course. *CBE Life Sci. Educ.* 12, 542–552. doi: 10.1187/cbe.11-08-0066

Staley, J. T., and Konopka, A. (1985). Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Ann. Rev. Microbiol.* 39, 321–346. doi: 10.1146/annurev.mi.39.100185.001541

Stevens, J. L., DeHority, R., and Goller, C. C. (2017). Using QIIME to interpret environmental microbial communities in an upper level metagenomics course. *CourseSource* 17:3.

Tan, T. W., Lim, S. J., Khan, A. M., and Ranganathan, S. (2009). A proposed minimum skill set for university graduates to meet the informatics needs and challenges of the "-omics" era. *BMC Genomics* 10:36. doi: 10.1186/1471-2164-10-S3-S36

Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., et al. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* 551, 457–463. doi: 10.1038/nature24621

Tobin, T. C., and Shade, A. (2018). A town on fire! Integrating 16S rRNA gene amplicon analyses into an undergraduate microbiology lecture class. *FEMS Microbiol. Lett.* 365, 1–197. doi: 10.1093/femsle/fny104

van de Guchte, M., Blottière, H. M., and Doré, J. (2018). Humans as holobionts: implications for prevention and therapy. *Microbiome* 6, 1–6. doi: 10.1186/s40168-018-0466-8

Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. A., et al. (2004). Environmental genome shotgun sequencing of the sargasso Sea. *Science* 304, 66–74. doi: 10.1126/science.1093857

Vision and Change in Undergraduate Biology Education (2011). *NSF and AAAS President's Council of Advisors on Science and Technology. Engage to Excel: Producing one Million Additional College gr Aduates With Degrees in Science, Technology, Engineer ing, and Mathematics. 2012*. Washington, DC: Vision and Change in Undergraduate Biology Education.

Wang, J. T. H. (2017). Course-based undergraduate research experiences in molecular biosciences-patterns, trends, and faculty support. *FEMS Microbiol. Lett.* 364:157. doi: 10.1093/femsle/fnx157

Weber, K. S., Jensen, J. L., and Johnson, S. M. (2015). Anticipation of personal genomics data enhances interest and learning environment in genomics and molecular biology undergraduate courses. *PLoS One* 10:486. doi: 10.1371/journal.pone.0133486

Williams, J. J., Drew, J. C., Galindo-Gonzalez, S., Robic, S., Dinsdale, E., Morgan, W. R., et al. (2019). Barriers to integration of bioinformatics into undergraduate life sciences education: a national study of US life sciences faculty uncover significant barriers to integrating bioinformatics into undergraduate instruction. *PLoS One* 14:288. doi: 10.1371/journal.pone.0224288

Wilson Sayres, M. A., Hauser, C., Sierk, M., Robic, S., Rosenwald, A. G., Smith, T. M., et al. (2018). Bioinformatics core competencies for undergraduate life sciences education. *PLoS One* 13:878. doi: 10.1371/journal.pone.0196878

Xie, S., Lan, Y., Sun, C., and Shao, Y. (2019). Insect microbial symbionts as a novel source for biotechnology. *World J. Microbiol. Biotechnol.* 35:25.

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Implementation of Antibiotic Discovery by Student Crowdsourcing in the Valencian Community Through a Service Learning Strategy

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Antibiotic misuse is a public health problem due to the appearance of resistant strains in almost all human pathogens, making infectious diseases more difficult to treat. The search for solutions requires the development of new antimicrobials as well as novel strategies, including increasing social awareness of the problem. The Small World Initiative (SWI) and the Tiny Earth (TE) network are citizen science programs pursuing the discovery of new antibiotics from soil samples and the promotion of scientific culture. Both programs aim to bring scientific culture and microbiological research closer to pre-university students through a crowdsourcing strategy and a Service Learning (SL) educational approach, with a 2-fold objective: to encourage students to pursue careers in science and to involve them in the discovery of soil microorganisms producing new antimicrobials. SWI and TE projects were put into practice in Spain under the common name *MicroMundo*. *MicroMundo*@Valencia was implemented at the Universitat de València (UV) during the academic years 2017–2018 and 2018–2019. It trained 140 university students to disseminate this initiative into 23 high/secondary schools, and one primary school, involving about 900 people (teachers and students) as researchers. A total of 7,002 bacterial isolates were obtained from 366 soil samples and tested for antibiosis at UV and high/secondary school centers. About 1 or 7% of them produced inhibition halos for the *Escherichia coli* or *Bacillus cereus* target strains, respectively. Geolocation of sampling sites by an application developed *ad hoc* and Kriging analysis also allowed detection of soil foci of antibiotic-producing bacteria. Evaluation of the project by university, high/secondary, and primary school students revealed their strong positive perception and their increased interest in science, as a consequence of acquiring new scientific and pedagogical concepts and skills that they were able to pass on to other classmates, younger students, or relatives. To further expand the dissemination of the project in the Valencian Community,

diverse extramural activities deemed to include a gender perspective and aimed at different age groups, were also carried out, obtaining very satisfactory results, increasing sensitivity and awareness to the global antibiotic crisis.

Keywords: antibiotic discovery, student crowdsourcing, antimicrobial resistance, service learning, soil bacteria, geolocation, *Natura* project, gender equality

INTRODUCTION

Arguably, antibiotics are one of the greatest discoveries of modern medicine and, during the first decade, carried away by the success, health professionals were not aware of the fragility of that medical breakthrough. However, the phenomenon of antibiotic resistance was known from the very beginning: Penicillin was introduced into clinical use in 1941, but its inactivation by an *Escherichia coli* strain with penicillin-hydrolyzing enzymatic activity had already been reported 1 year before (Abraham and Chain, 1940) and by 1942, penicillinase-producing strains of *Staphylococcus aureus* were found in hospitalized patients (Rammelkamp and Maxon, 1942). It could then be said that antibiotics, in a way, carried the potential for their own downfall. To make matters worse, the misuse (for either unnecessary, inappropriate, or both), the overuse of antibiotics, and the improper level of microbial exposure (mode and timing of administration, dose, dosing interval, etc.) when treating patients over the last almost 80 years, have taken us to the brink of losing the war against bacterial pathogens.

The outcome of all these factors is the situation we face today, in which the emergence of antibiotic-resistant strains across almost all human pathogens is wreaking havoc (Burnham et al., 2019). Undeniably, the solution not only requires the development of new antimicrobial drugs acting through new mechanisms and aiming to new targets (Mulani et al., 2019; Wang et al., 2020) but it also demands additional new strategies able to pave the way to move forward, like the establishment of adequate antibiotic stewardship policies within the medical community as well as raising social awareness of the magnitude of the problem we have to tackle.

This is where initiatives such as the Small World Initiative (SWI), a citizen science project pursuing to discover new antibiotics and promote scientific culture, that originated in 2012 at Yale University (United States; Hernandez et al., 2015), or the Tiny Earth (TE) network, an educational program headed by the UW-Madison's Wisconsin Institute for Discovery (United States), initiated in 2018, that strives to forge a network of instructors and students focused on student-sourcing antibiotic discovery from soil, may become crucial when it comes to raising the much needed community awareness. Both proposals address the pandemic spread of antibiotic resistance by targeting at individual-level two of its structural roots, namely, the current shortage in the development of new therapeutic antimicrobial drugs and the lack of awareness and understanding of the problem within the general population.

Both programs aim to bring scientific culture and biomedical research closer to pre-university education levels through a crowdsourcing strategy, aimed at discovering novel microorganisms producing new antibiotics from soil samples. Emulating the discovery of penicillin by Alexander Fleming, but in an organized and participatory way, the programs seek to involve a large group of volunteers or a community, through an open call, with a 2-fold objective. On the one hand, they aim to encourage students to pursue careers in science, as well as to entice them to become the scientists of tomorrow (Caruso et al., 2016; Davis et al., 2017). In addition, by involving citizenry in the discovery process, they greatly increase the chances of finding new bioactive molecules, which might prove to be useful therapeutics. Both initiatives are currently underway at different educational levels, in at least 15 countries and involving tens of thousands of students.

To engage students in the SWI and Tiny Earth research experiences in microbiology, the innovative educational strategy Service Learning (SL; Esson and Stevens-Truss, 2005; Webb, 2017) was used. The SL is an educational proposal that develops both learning procedures and community service in a single project since, by connecting motivation with experimentation, the training of the students is enhanced, and they in turn are involved in the real needs of their close environment with the aim of improving it. This learning strategy brings many pedagogical and social benefits to the students involved, improving their self-learning, academic and civic engagement, social skills, and moral reasoning (Esson and Stevens-Truss, 2005; Celio et al., 2011; Lies et al., 2012; Webb, 2017).

In July 2017, the SWI@Spain network was created at the *Universidad Complutense de Madrid* (UCM), sponsored by the group of Teaching and Dissemination of Microbiology (D+DM) of the Spanish Society for Microbiology (SEM; Valderrama et al., 2018), in which SWI@Valencia from the *Universitat de València* (UV) participated as a foundational member.[1] Currently, 31 groups are working in different universities from the Iberian Peninsula (Spain and Portugal), under the common name *MicroMundo*, joining the efforts of nearly 100 instructors and an even higher number of secondary education schools.

The *MicroMundo*@Valencia initiative was implemented at the UV in the academic course 2017–2018, similarly to SWI@Spain/*MicroMundo* at the UCM (Valderrama et al., 2018). Likewise, the main objectives were: (i) to generate sensitivity toward antibiotic resistance and the need to spread awareness of the problem to the society at different educational levels, (ii) to awaken the interest in science through experiential learning and student discovery of bacteria that produce new antibiotics, and (iii) to encourage scientific training and learning in science by mutual cooperation among students of different education levels through a community service.

[1]http://swispain.blogspot.com/2017/07/i-workshop-swispain-2017-el-programa.html

In the 2018–2019 course, *MicroMundo*@Valencia also traveled to primary schools, within the framework of the "*Natura* projects" program of the UV. This innovative program tries to unite the work done by different natural science projects and to improve the teaching quality using SL as educational strategy by training students of different educational levels scientifically through adapted sessions. The interest of the project resides in the fact that teaching is given by university students to high school students and subsequently by the latter to primary school students. It is a good educational tool since university students reinforce their knowledge by being responsible for transmitting it to high/secondary school students, while the latter learn new concepts that can also be transmitted to primary school students. Thus, the project promotes the connection of university students with those of high/secondary and primary schools which offers a unique opportunity for social and intellectual interactions between them hardly achievable in other ways (Abrahamsen, 2004). Moreover, the implementation of *Natura* projects in the different educational levels, implies the extension and complementation of the curricular content within the subjects of biology and geology for the high/secondary school level, and of natural sciences in the case of primary education.

Finally, to further extend the dissemination of the *MicroMundo*@Valencia initiative into the society, different extramural activities were carried out including participation in scientific fairs, gender equality, and infographic activities for high/secondary school students, scientific meetings, as well as in diverse community-wide actions to achieve significant media coverage. In this paper, we report the results of our participation as the *MicroMundo* group in Valencia's Community, as well as in additional outreach activities outside the UV.

MATERIALS AND METHODS

Implementation of *MicroMundo*@Valencia at the UV

Professors from the Departments of Microbiology and Ecology, Soil Science, and Computer Science of the UV, as well as other members of the Department of Microbiology and Ecology were involved in the project implementation. Microbiology professors (*MicroMundo* Partner Instructors, MMPIs) carried out the recruitment of university undergraduate students of microbiology of the different degrees and masters of the UV and taught them a training course to become monitors (*MicroMundo* Teaching Assistants, MMTAs; see below). MMPIs were also in charge of contacting, recruiting, and selecting the participating partners (public high/secondary education schools), as well as in obtaining funds from different UV sources. Science secondary teachers from public schools involved in this initiative also participated applying for funds to the local government. Additionally, in the context of the "*Natura* projects" program at UV, some MMPIs, one MMTA, and a group of high school students (see below) participated in a pilot project to adapt and transfer *MicroMundo*@Valencia to primary schools.

A scheme of the progressive implementation of *MicroMundo*@Valencia at different educational levels in the Valencian Community is shown in **Figure 1**.

Biosafe Practices

Since activities of this project involve working with live microorganisms, biosafety practices for teaching laboratories (Emmert, 2013) were adopted and taught to the students to safely work both, in Microbiology laboratories at UV and in science laboratories at schools, as well as to prevent the potential spread of the microorganisms. All students had disposable lab coats and gloves, and laboratory benches or classroom tables were disinfected both before and after each practical session. Further, the waste material derived from each session was collected and autoclaved at the UV facilities. Prior to science practices, all school students were authorized by their respective centers and parents or tutors.

Training Courses for Monitors

Initially, a call was made to the students of different science degrees (Biochemistry and Biomedical Science, Biology, Biotechnology, Environmental Sciences, Food Science and Technology, and Pharmacy) and Masters (Molecular, Cellular and Genetics Biology, Secondary Education Teaching, and Food Quality and Safety) enrolled at the UV, as well as in the Master in Social Communication of Scientific Research at the *Universidad Internacional de Valencia*. The basis of the project was explained in a presentation talk, which was updated and exposed annually at the beginning of each course to attract new undergraduate students to the *MicroMundo*@Valencia team. Due to the large number of students who applied to participate in the project, a selection was made

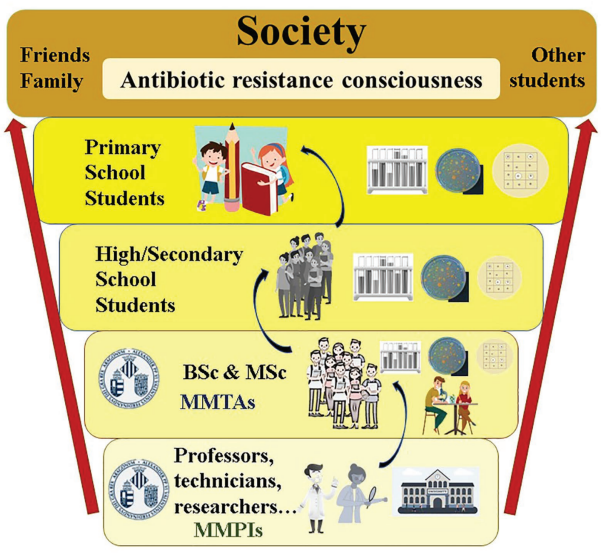

FIGURE 1 | Scheme of progressive implementation of *MicroMundo*@Valencia at different educational levels, from university to primary school, in the Valencian Community.

in order to carry out their training, in a coordinated manner, with the Microbiology MMPIs in three laboratories of the Faculty of Biological Sciences at UV, each academic year. MMTAS were trained through five theoretical-practical sessions in laboratories with the support of a manual adapted from Hernandez et al. (2015) and Valderrama et al. (2018). Briefly, these practical sessions included: (1) explanations on bacterial resistance, soil sampling, and use of a new geolocation application; (2) diluting and plating soil samples for isolation of microorganisms; (3) observation of soil microbial diversity, plate counts, and selection of colonies; (4) testing soil microorganisms for antibiosis activities; and (5) antibiosis observation, results interpretation, and surveys. The five sessions were similar to those held later in the schools, adapting the contents (see below). Both the educational and laboratory materials for the science practices in secondary schools were prepared by the MMTAs under the supervision of their corresponding MMPIs.

Science Practices in High/Secondary Schools

Basically, the project consisted of five sessions lasting around 2 h separated by at least 2 days to allow the growth of soil microorganisms at room temperature. The sessions took place in the science laboratories of each one of the high/secondary schools participating in the project. Culture media for bacteria, sterile containers, and other materials were provided by the *MicroMundo*@Valencia teams. Students were also provided with an edited booklet with instructions. The content of each session is summarized below.

Session 1: Explanations on Bacterial Resistance, Soil Sampling, and Use of a New Geolocation Application

This first session started with the presentation and moderation of a debate on the problem of bacterial resistance to antibiotics and its socio-economic impact. For this, the different *MicroMundo*@Valencia teams used the same presentation in all high/secondary schools. This presentation also included a step by step description of the methodology. Each working group was made up by one MMPI and three to five MMTAs. Sampling material and instructions on how to choose a good sampling place were also provided at this session to groups of two or three students, which was normally programmed on a Friday to allow students to take a trip over the weekend to different places of Valencian Community to get soil samples. A mobile phone application specifically developed for this project downloaded the coordinates of the sampling site into a database. Further data related to encompassing features of the environment were provided by students through a fill-in form. Sterile containers were provided for both the microbiology and edaphology samples. Instructions were also provided on the important matter of the identification of the samples, using a code identifying the high/secondary school and the university team responsible for the sample, as well as for storage at room temperature.

Session 2: Diluting and Plating Soil Samples for Isolation of Microorganisms

Once edaphology samples were collected, work was started on the isolation of soil microorganisms from the microbiology samples. To this end, 9 ml of sterile water was added to 1 g of soil and serial 10-fold dilutions up to 10^{-5} were performed. One hundred microliter of the corresponding dilutions was spread on the surface of trypticase soy agar (Insulab, Valencia, Spain) diluted 1/10 (TSA 1/10, with agar up to 1.5%) plates using a Digralsky spreader and the plates were left to incubate at room temperature in a dark place. The addition of cycloheximide to TSA was ruled out due to the toxicity of this fungicide. In all this work, the secondary students were supervised by the MMTAs.

Session 3: Observation of Soil Microbial Diversity, Plate Counts, and Selection of Colonies

After 3 days of incubation, plates were inspected for having either well isolated colonies or between 20 and 200 colonies for plate counts. Individual colonies were transferred to fresh full-strength TSA plates using sterile toothpicks. Up to 20 individual colonies were transferred to a single plate placed over a grid template enabling easy colony coding and identification.

Session 4: Testing Soil Microorganisms for Antibiosis Activities

TSA plates were inoculated to saturation with a standard Gram positive bacterium, *Bacillus cereus* CECT 101, or a standard Gram negative bacterium, *E. coli* CECT 495, and left at 4°C overnight in the UV laboratory to prevent growth, before taking them back to the secondary schools. Students transferred the colonies isolated in the previous session onto the growing lawns of *E. coli* or *B. cereus* under the supervision of the MMTAs. For this, sterile toothpicks were used and the plates were placed on top of the template used in the previous session so that the distribution of the soil microorganisms on these plates matched the original distribution of their first isolation.

Session 5: Antibiosis Observation, Results Interpretation, and Surveys

After 2 days of incubation at room temperature, the plates were observed against a strong backlight in search of growth-inhibition halos identifying antibiosis phenomena. An interpretation of the results was made with the help of the MMTAs. Comparisons on the different effect of the same soil microorganism on Gram positive and Gram negative bacteria were made on the context of the spectrum of the possible antibiotics produced. The most promising soil microorganisms were transferred to fresh TSA plates to obtain pure culture for further characterization and study. Evaluation questionnaires were distributed to secondary school teachers and students and to MMTAs, and feedback was given to school teachers to prepare posters and videos for the closing day of the project (see below). Finally, all school students and teachers received a diploma to recognize and acknowledge their contribution.

Science Practices in Primary Schools

Although the *MicroMundo*@Valencia project has traditionally focused on high/secondary education level, in the academic course 2018–2019, it was implemented in students of a local primary school through a *Natura* project at UV in order to spread awareness of the problem of antibiotic resistance from high/secondary school to elementary level. The thematic blocks related to the project for high/secondary education within the subjects of biology and geology were "Cell organization," "Biodiversity," and "Scientific methodology." In the case of primary education, the blocks within natural sciences were "Human beings and health" and "Living beings." Within each block, students could acquire and practice key competencies including, among others, language communication abilities, digital capacities, mathematical and basic skills in science and technology, as well as social and civic aptitudes.

Two classes of sixth grade of primary education (10–11 years old) of the school CEIP San Juan de Ribera (Burjassot, Valencia) participated in the project together with a group of high school students from the Vicent Andrés Estellés high school (Burjassot, Valencia). The high school students were trained by a MMTA, under the supervision of three MMPIs, to act as monitors for primary students through adapted sessions. For this purpose, four sessions were planned, the first two focused on the training of the high school students at their school, and the last two on the practical classes for the elementary students at their school. The practical classes were more approachable than those at the high school and adapted to this level (10–11 years old). The sessions are briefly explained below, summarizing their contents.

Session 1: Explanation About the Project

During this first session at the high school, *Natura* project was explained to high school student volunteers and recommendations about their role as instructors were made so that they could adapt the project contents to primary students level.

Session 2: High School Students' Exposition and Evaluation

In this case, the powerpoint presentations and questions related to the project prepared in advance by the high school students were revised to check their suitability for the elementary students. These questions were prepared to assess the degree of understanding of these younger students about the new concepts and knowledge explained.

Session 3: Practical Class 1. Introduction to the Project. Culturing Soil Bacteria

This session took place at the primary school with groups of four to five students. First of all, the high school students explained to the younger ones the main important concepts to follow the rest of the activities, as well as biosafe measures to work with microorganisms. After that, students proceeded with the practical part with all the material being supplied by *Natura* project and participating MMPIs. Dilutions of soil samples were provided as well. Primary school students used a sterile swab that was introduced in one of the dilutions tubes and thereafter spread on the surface of one TSA plate, in the same way as it was done by MMTAs and high school students in the *MicroMundo*@Valencia project.

Session 4: Practical Class 2. Microbial Diversity and Antibiosis Observation

After 1 week of incubation, primary school students were able to see the bacterial diversity coming from the soil samples used. They observed diverse bacterial colonies on TSA plates, and were able to see natural antibiosis phenomena that some of them produced against other microorganisms present in the same plate. Moreover, and to complement the activities and the information given to the students, antibiosis assays against one strain of *B. cereus* were prepared at UV using bacterial colonies of the same soil samples. Plates with growth inhibition halos were shown to the elementary students in order they saw the microbial diversity in a soil sample, the antibiosis phenomenon among some of the soil bacteria, and the antibiosis against a specific bacterial strain. Thereafter, some questions were made to the primary students to favor their knowledge acquisition related to the project. Finally, high and primary school students and teachers received a diploma as an acknowledgment for their participation.

Evaluation of MMTAs/Students and Science Practices

At the end of the practical sessions at the UV and the schools, anonymous surveys were filled by MMTAs and high/secondary and primary school students and teachers, adapting the questionnaire according to the involvement and educational level. Surveys presented different blocks and allowed responses based on a 5-value Likert scale, with 1 being the most negative and 5 the most positive (Likert, 1932). This scale was used to try to measure the attitudes, opinions, and perceptions of the different participating students regarding the problem of antibiotic resistance, how some of them could help to explain the problem to younger students and teach them practices on discovery of soil bacteria potentially producing antibiotics, and whether the accomplishment of these practices can improve their knowledge and awareness of a real problem, as well as increase their interest in science. In addition, an open block was included to provide opinions and make proposals for the improvement of project activities. Due to the anonymous nature of the surveys, the results could not be separated according to the gender of the students.

MicroMundo Teaching Assistants surveys consisted of four closed blocks to assess their opinion on the scientific interest of the project, stress the concept of antibiotic resistance, and expose their experience as teachers for younger students and on their collaboration in the project. The MMTAs survey was based on that of Valderrama et al. (2018) which, in turn, was based on the United States SWI Undergraduate Research Student Self-Assessment document (URSSA, SWI, United States). Surveys for high/secondary school students were simpler, with three closed blocks of questions on their scientific interest, their previous knowledge about antibiotic resistance, and their participation in the project. The secondary school teacher surveys consisted of

two closed blocks on the interest of the project for their students and their personal opinion. Primary school student surveys were even shorter, with only six questions distributed in three blocks about their learning experience, their interest in the project, and their opinion about the high school monitors.

Computer Science

An Android application (APP) was designed and developed to geolocate the collection sites of the soil samples, filling a form to characterize the site, and labeling each sample. This APP is available at the link https://www.uv.es/swi/swi.apk. Once downloaded and installed on the phone or tablet, the APP sends the information gathered with a form asking for the environmental conditions of the sample collection and it is sent using POST protocol to a spreadsheet in a Google server that gathers this information to be processed later. A guide was written to facilitate the use of the application as well.[2]

Also, the geolocation of the samples was used to draw a map of sampling sites, in order to be able to show all the information available from the form. The aim was that the processing of the samples provided information on the microorganisms able to inhibit the growth of control bacteria (*E. coli* and *B. cereus*). With this information, different maps were developed using spatial statistics techniques, such as Kriging technique (Wackernagel, 2003), in order to show the spatial statistical presence of these soil bacteria.

Soil Science

Since it was important to know the characteristics of the soil and its environment in order to look for possible relationships with the production of antibiotics by soil microorganisms, a group of MMTAs participated in the soil session "Easily identifiable soil characteristics and their influence on its microbial inhabitants." The goal was to learn the importance of the soil as a resource and identify some characteristics that may influence the type of soil microorganisms. The students worked in the Department's Edaphology laboratory with the soil sample they had taken to do the *MicroMundo*@Valencia practice in the Microbiology laboratory. The size and number of the species diversity of soil microorganisms depend on various factors, mainly on the physical and chemical soil characteristics such as texture, humidity, pH, and organic matter content (Voroney, 2007; Mansour et al., 2015). These soil properties were determined according to the official laboratory methods of the Ministry of Agriculture, Fisheries, and Food (MAPA, 1994).

Closing Day

All school students involved in the *MicroMundo*@Valencia project were invited to prepare posters, videos, or presentations about their results to show them in the closing day organized by the MMPIs at UV. The event started with the presentation of different political and academic authorities contributing to the project. Afterward, different activities were run, photocall, poster, and video competitions for the high/secondary school students as well as a Kahoot competition[3] organized by MMTAs and MMPIs to reinforce students learning consisting of asking questions with multiple answers about the project. Finally, students and teachers and MMTAs received diplomas and awards for their contribution.

Extramural Dissemination Activities

Science Fair *Expociencia*

A group of MMTAs and MMPIs attended every year the *Expociencia* science fair, promoted by the *Parc Científic* of the UV, held on the last Saturday of May. The aim was to show the project to all education levels using this platform. A multiple workshop consisting of two sections, an exhibition and another for handling samples, was designed. The exhibition workshop consisted of 5-min sessions, adapted to the age of the audience (children, teenagers, or adults) and showed the importance of the good use of antibiotics, as well as the present and future dangers of not doing so. Different explanatory panels adapted to the audience were prepared. In the second part, children and young people swabbed samples on TSA 1/10 plates. Participants were given a bookmark with a link where photos of the sown plates could be viewed later. Adults received an information card (business card format) to request additional information/visits to schools/research institutes if desired.

Activities in Favor of Gender Equality

A priority aim was to disseminate the project both at university and pre-university levels with a perspective of gender equality, taking advantage of the initiative #11 February (International Day of Women and Girls in Science). Under this scenario, university female students gave talks in high schools in the Valencian Community explaining the project, in terms of both its scientific basis and the importance of their own participation as scientists under training. After a presentation session, a debate with the speaker ran, asking questions about her experiences. The objective was 2-fold, to awaken scientific interest in the proposal and to motivate undergraduate students to choose science, technology, engineering, and mathematics (STEM) careers, since the analyses of the future labor market offer interesting perspectives of professional development for students of these degrees (Education and Training Monitor EU analysis, 2019).

Meeting on Teaching and Dissemination of Microbiology

Selected posters from the closing day and others, as well as oral communications based in the project or results obtained at the UV, were prepared by MMTAs under the supervision of their MMPIs, and were presented in the meeting of the D+DM group of the SEM in July 2018 at the UCM in Madrid (Spain).

Outreach Conferences

Conferences, round tables, and other activities were held in high/secondary schools located in the Valencian Community

[2]https://www.uv.es/swi/manualapk.pdf

[3]http://kahoot.com

(in a radius of 150 km from UV) under the suggestion of the Scientific Culture and Innovation Unit Chair for Scientific Dissemination of UV. Lectures were followed by question/answer sessions in which students and teachers participated. In addition, training sessions were organized for secondary school teachers, also participating in monographic conferences in the Science Weeks running in towns of our Community or conference cycles in singular scientific centers (Institute of Biomedicine of Valencia, City of Arts and Sciences of Valencia, Planetarium of Castellón).

DivulSuperBugs

A group of students from the Biology degree at the UV prepared several infographics with information on the antibiotic crisis, antimicrobial resistance, and superbugs under the supervision of one of the participant professors. Infographics were submitted to a peer review system, where errors were reviewed and more information was provided when necessary. The following step was to establish teaching relationships among the infographics and create a cross-linked system to shape them as an exhibition. Two to three UV students acted as monitors of the exhibition in each school. Firstly, a brief explanation was given to the secondary school students and they could visit the infographics over a period of 1 week. Finally, direct learning was assessed using game-based classroom quizzes which engage students (Kahoot, escape rooms, etc.) designed by the monitors. Finally, anonymous surveys were run to evaluate the activity.

Media Coverage

The target audience of the previous activities was mainly adolescents. People in higher age ranges were accessible through other channels, such as conventional television, radio stations, or newspapers. In order to bring the problem of antimicrobial resistance closer to these age groups, specific collaborations were started with different public and private broadcasting companies.

A summary of the different SL and dissemination activities carried out for the implementation of *MicroMundo*@Valencia in the Valencian Community is shown in **Figure 2**.

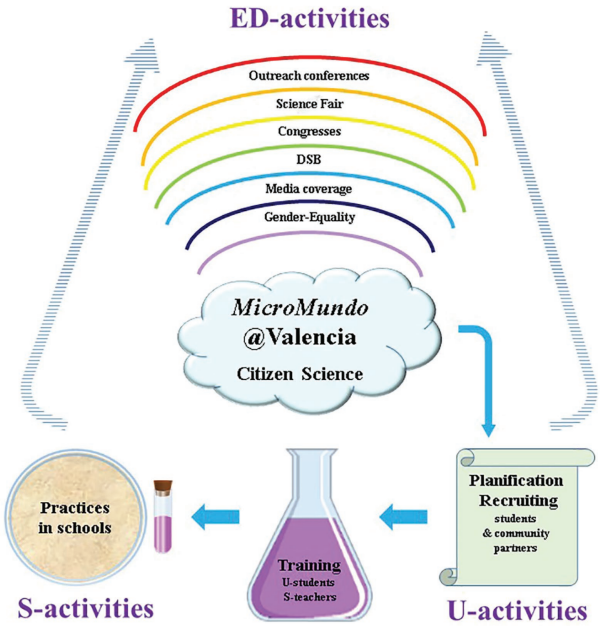

FIGURE 2 | Flowchart of the different Service Learning and dissemination activities for the implementation of *MicroMundo*@Valencia in the Valencian Community. ED, extramural dissemination; S, school; U, university. Project activities in the Valencian Community began at the Universitat de València (U-activities) where university students (U-students) and school teachers (S-teachers) were recruited and trained to later adapt the activities to high/secondary and primary schools (S-activities). Project's university and school activities were further disseminated in the Valencian Community through the implementation of diverse extramural activities (ED-activities) aimed at different age groups and with a gender perspective. Results, analyzed at the university, showed an increased sensitivity and awareness of the Valencian society to the global crisis of antibiotic resistance.

RESULTS AND DISCUSSION

Service Learning in microbiology courses is a teaching methodology that involves the participation of university and school students in research with the aim to improve their education and science interest as well as the community's health (Webb, 2017). Two research-based programs focused on student discovery of novel soil microorganisms potentially producing new antibiotics, SWI, and TE, started in Spain in 2017 and 2018, respectively, under the current common name *MicroMundo*.

The implementation of the *MicroMundo* project at the UV in the Valencian Community during the academic courses 2017–2018 and 2018–2019, allowed us to train up to 140 university students to disseminate the project to 23 high/secondary schools (**Figure 3A**) and one primary school in our influence area, involving some 900 people (teachers and students; **Table 1**). Particularly, in the course 2017–2018, 300 students and 14 teachers from 10 secondary schools from a network of public centers of the Valencian Community participated in *MicroMundo*@Valencia, as well as 66 MMTAs and 15 MMPIs from the UV. In the following academic course, 314 students and 17 teachers from 13 secondary schools were involved, together with 73 MMTAs and 10 MMPIs from the UV. The project also approached to elementary school in 2018–2019, involving 42 students (21 per classroom) and 2 teachers from a public primary school, 14 students and 1 teacher from a public high school, and 1 MMTA and 3 MMPIs from the UV.

Sample Collection and Isolation of Soil Microorganisms

The collection of soil samples and the isolation of microorganisms from these samples by the MMTAs and the students of the participating schools were performed in the first and the second semesters, respectively, of each academic year. In the case of MMTAs, samplings were done during the training course in November 2017 and November 2018, with three groups of 22–24

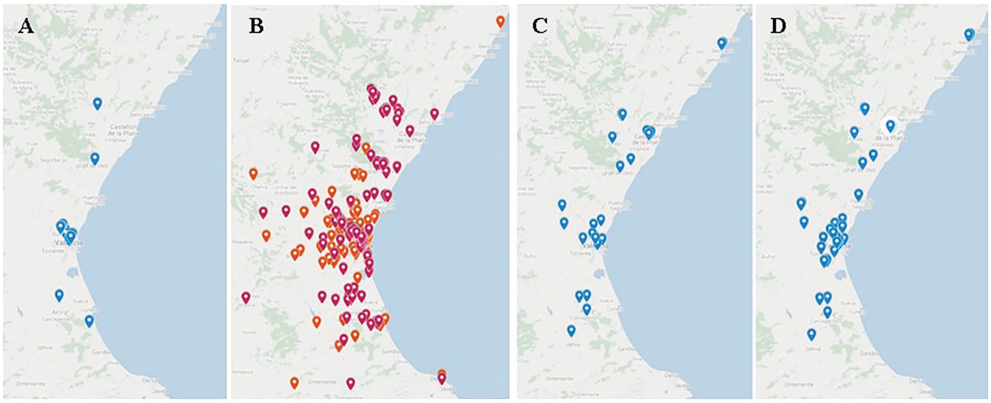

FIGURE 3 | Location maps of some of *MicroMundo*@Valencia activities in the Valencian Community. **(A)** Discovery program in secondary schools (23). **(B)** Sampling points 2017/2018 (173) orange and 2018/2020 (193) pink. **(C)** Gender quality activities (30). **(D)** Conferences and weekly expositions (43).

TABLE 1 | Professors and students involved in *MicroMundo*@Valencia initiative at the *Universitat de València* in the Valencian Community.

Academic course	MMPIs	MMTAs	Schools	Schools teachers	MMSs	Primary students
2017–2018	15	66	10	14	300	
2018–2019	10	73	13	17	314	
2018–2019 *Natura* project	3	1	1	3	14	42
Total	28	140	24	34	628	42

students each year. In the case of the school students, samplings were carried out along the months of February to April in 2018 and in 2019. During the first and second academic years, 173 and 193 soil samples were collected, respectively (**Table 2**). Samples were taken from soils of different geographical locations of the Valencian Community that were geolocated. The locations in 2017–2018 and 2018–2019 are presented in **Figure 3B**. Most soil samples were collected at a depth of between 0 and 2 cm (**Figure 4**), normally at temperatures ranging from 1 to 19°C in 2017–2018 and between 6 and 18°C in 2018–2019.

Microbial counts were performed to estimate the heterotrophic bacteria in each one of the soil samples (**Figure 4**). Plate counts were carried out in a medium commonly recommended for the isolation of soil bacteria, diluted TSA containing only 1/10 of the nutrients (TSA 1/10; McCaig et al., 2001; Joseph et al., 2003), to favor the isolation of facultative oligotrophic bacteria from nutrient-poor environments such as soil. This medium was the same used previously by Valderrama et al. (2018) in SWI@Spain except that in our case it was not supplemented with the fungicide cycloheximide, since colony counts with bacterial morphology were performed after 72 h at room temperature. In our conditions, the plate counts of aerobic mesophilic heterotrophic bacteria obtained from the soil samples ranged from around 10^5 to 10^7 colony forming units per gram (CFU/g).

Since edaphic factors including pH, organic matter, texture, and carbonate contents, among others, influence microbial abundance (Mansour et al., 2015), some soil characteristics of a selection of samples were studied. The results revealed that most of the Mediterranean soils analyzed presented brownish coloration, sandy texture, and slightly alkaline pH values (7.2–8.5). Moreover, darker soils showed a higher content in organic material and, therefore, a higher content in microorganisms (Zhang et al., 2014). Soil samples with clay-like texture were those with higher number of CFU/g. These results agree with previous studies showing that cell numbers and microbial biomass are most concentrated in the smaller size silt and clay soil fractions (Sessitsch et al., 2001), which can be related with the influence of soil texture on nutrient retention and availability (Silver et al., 2000).

Soil isolates were tested by MMTAs or high/secondary students for their potential antibiosis activity against one standard Gram negative bacterium, *E. coli* CECT 101, and one standard Gram positive bacterium, *B. cereus* CECT 495. In the 2017–2018 course, from a total of 3,142 soil isolates tested, 73 (2.3%) showed growth inhibitory activity against *E. coli*, while 194 (6.2%) were active against *B. cereus* (**Table 2**; **Figure 4**). In 2018–2019, from 3,860 isolates tested, 10 of them (0.2%) were positive showing growth inhibition halos around the *E. coli* strain, whereas 278 (7.2%) showed inhibitory response against the strain of *B. cereus*. Taking into account the results of the antibiosis assays of the two courses together, from a total of 7,002 soil isolates tested, the percentages of isolates active against the *E. coli* or *B. cereus* strains were 1.2 or 6.4%, respectively. Only very few strains were active against both Gram-positive and Gram-negative bacteria. Although it is difficult to compare with the previous results of SWI@Spain by Valderrama et al. (2018) and de Groot et al. (2020) given that the number and type of soil isolates tested, as well as the control strains used for the antibiosis assays were different, the present results are similar in the sense that a higher percentage of soil strains with antimicrobial activity against Gram positive bacteria than Gram negative was obtained. In our case, the percentage of positive bacterial strains was higher than in the two previous studies which could be due, at least in part, to the higher number of soil isolates tested. This large number of soil isolates obtained as well as the relatively high percentage of them with potential activity against Gram positive

TABLE 2 | Soil samples analyzed and results of *MicroMundo*@Valencia initiative at *Universitat de València* and high/secondary schools.

Course	Samples	Isolates	Antibiosis G−	Antibiosis G+
2017–2018	173	3,142	73	194
2018–2019	193	3,860	10	278
Total	366	7,002	83	472

or Gram negative bacteria has been made possible through the *MicroMundo*@Valencia initiative in which a large number of people have participated.

Based on our results, the antibiosis tests at the primary school with a total of 320 soil isolates, after adapting the assay, were only carried out against the strain of *B. cereus* (**Figure 5**). In this case, 5.3% of soil isolates tested (17 out of 320) was positive showing an inhibitory halo of growth around the *B. cereus* strain. Thus, all our results confirmed that, similar to Valderrama et al. (2018) and de Groot et al. (2020), it was easier to find soil microorganisms able to inhibit Gram positive bacteria such as *B. cereus* than Gram negative bacteria such as *E. coli*. In this sense, it is expected that the soil bacteria tested show antibiosis activities against other soil bacteria such as *B. cereus* against which they may have to compete for space and/or resources to survive in microbial communities (Hibbing et al., 2010; Stubbendieck and Straight, 2016). Besides, it is more difficult to find soil bacteria producing antimicrobial substances against *E. coli*, whose natural habitat is the intestine. In addition, the outer membrane of Gram-negative bacteria is a semi-permeable barrier that protects them against different toxic compounds, including antimicrobials (Miller, 2016).

The antibiosis activity observed with some of the soil isolates was later confirmed in microbiology laboratories at UV. Those isolates that maintained their activity after purification were stored at −80°C for further studies since the two control bacteria used for the antibiosis tests were safe relatives to the bacterial pathogens grouped under the acronym "ESKAPE" (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.). ESKAPE bacteria are threatening nosocomial pathogens because of their resistance to most of the antimicrobial agents (Rice, 2008; Navidinia, 2016). In fact, they are in the World Health Organization (WHO) priority list of antibiotic-resistant bacteria against which new antimicrobials are needed (Tacconelli et al., 2018).

On the other hand, the geolocation of the soil samples allowed to draw a map of sampling sites (**Figure 6**), showing all the information available from the data collection form. With this information, different maps using Kriging technique were developed in order to show the spatial statistical presence of these bacteria. **Figure 6A** shows a map with the CFU/g as a colony density parameter of the elements that were available in each sample and interpolated to the metropolitan area of Valencia, not only the sampling points. This knowledge enables us to estimate in which areas we can find a higher level of bacteria and, later on, bacteria producing antibiotics. Further, the processed soil samples allowed gathering information of microorganisms able to inhibit the growth of control bacteria (*E. coli* or *B. cereus*). Thus, by performing a Kriging analysis, five soil foci that allowed the isolation of soil microorganisms able to inhibit the growth of *E. coli* and three foci of those able to inhibit the growth of *B. cereus* were detected (**Figures 6B,C**). These results suggest a potential application of geomapping to optimize antimicrobial research across large geographical regions to locate and predict the presence of antibiotic-producing microorganisms against *E. coli* and/or *B. cereus*.

Finally, the closing day of the *MicroMundo*@Valencia project was held in mid-May in each of the academic course in which it was carried out, to gather all the participating teachers and students. The objectives were to emphasize again the problem of antibiotic resistance by giving an informative talk, to show a summary of the results obtained in the project thanks to the effort made by all, and to promote the preparation and dissemination of results obtained through different activities and by conducting various competitions. As a result, a total of 13 videos, 25 posters, and 48 photos were presented to the assistants and numerous diplomas and prizes were awarded to the different participants.

Evaluation of Activities at the University

The *MicroMundo* Teaching Assistants' perception of the *MicroMundo*@Valencia project based on the analysis of all surveys carried out in two academic courses was very positive, as most of the questions asked obtained the highest scores (4 and 5) on a 5-value Likert scale (**Supplementary Material**). The results, grouped into the four blocks of the questionnaire, are shown in **Figure 7A**. In relation to the scientific interest of the project, 77.8% of the students answered with 5 (37.1%) and 4 (40.7%), respectively, that their participation in the project increased their interest in science. Likewise, a 77.8% of them, with 5 (37.8%) and 4 (40%), respectively, also considered that their results could contribute to the scientific progress. It is noteworthy that around 90% and more than 80% of MMTAs (with scores of 4–5 points in both items), expressed that the project was targeting a real problem and allowed them to learn about microbial diversity in the environment, respectively. With regards to antibiotic resistance, all students considered that the project contributed to a better understanding of bacterial resistance to antibiotics, with 5 (71.4%) and 4 (27.1%) points, respectively. Most of them (80.8%), with 5 (57.3%) and 4 (23.5%) scores, also expressed that the experience improved their awareness of antibiotic use. Remarkably, survey results on MMTAs experience as teachers for high/secondary students in the schools were also very high, since from 57 to 68.8% of them gave 5 points in the four items. When rating 4–5 points together, 95.7% of the MMTAs knew how to answer the questions asked by the younger students during the science practices and 90.7% expressed that they were able to respond to unplanned situations. Similarly, 90 and 91.4% of university students (jointly recording scores 4 and 5) considered that practical and theoretical explanations, respectively, were carried out in a way that school students were able to understand. Regarding MMTAs' opinion on their collaboration in the project

FIGURE 4 | Implementation of *MicroMundo*@Valencia initiative at high/secondary schools in the Valencian Community. From left to right and from top to bottom representative photographs are shown about project activities, including soil sampling, diluting and plating of soil samples for isolation of microorganisms, observation of soil microbial diversity, and selection of colonies to test their antibiosis activities and results interpretation. Except for samplings that were carried out independently by the different groups of students over the weekend, the rest of activities were performed in the science laboratories of their respective centers. For further details, see "Materials and Methods" section.

in the remaining five questions of the block of "other questions," it was very satisfying for more than 92% of them (rating 4–5 points in the five questions). In detail, three questions about whether the project had contributed to improve the MMTAs' scientific training, their transferable competences, or if they would recommend participation in this project to other students, reached scores of 5 points in 64.7, 61.9, and 69%, respectively. Regarding the global assessment of students' experience working on a real problem, the percentage of students that gave it a score of 5 to 4 was 47.8 and 44.2%, respectively. The last question in which the student had to reflect their global opinion on their participation in the project revealed very positive perception by MMTAs' with 5 (48.1%) and 4 (45.7%) scores.

Other questions with free answer were made to the students that took part in the project in order to know their opinion about the best and worst things related to the activity and recommendations to improve. Regarding to the best things, MMTAs considered that it was a gratifying activity, since they learned new concepts and did a type of training different from the practices they did during their studies. Furthermore, they were pleased to transmit all these knowledge and experience to high/secondary students and to inform them and the rest of the community about the problem on antibiotic bacterial resistance. Another important point is that they were satisfied to see high/secondary students enjoying and learning doing a scientific practice, which could encourage them to follow scientific careers. Some MMTAs pointed out that it was a good experience to act as teachers for younger students. Conversely, among the worst things the MMTAs considered that some of the practical sessions at the high/secondary schools were overlapping with their classes at the university, so they had to lose some of them. Other things they complained were about the material shortage or that the sessions with high/secondary students were too short. Therefore, these MMTAs made some recommendations to improve project activities. They suggested shortening the length of the formation sessions and to use more and diverse materials, for example, different culture media and different bacterial strains.

Evaluation of Activities in High/Secondary Schools

Survey results on students from high/secondary schools from two academic courses showed that they also liked the project activities. On a 1–5 scale, considering jointly the responses

FIGURE 5 | Implementation of *MicroMundo*@Valencia initiative at primary school within the framework of the *Natura* programme of the *Universitat de València*. In this innovative Service Learning program, teaching is given by university students to high school students and subsequently by the latter to primary school students, through adapted sessions, in their respective centers. Representative photographs of the activities of the project, including explanations and science practices (culturing soil bacteria and observations of microbial diversity and antibiosis). For further details, see "Materials and Methods" section.

with scores of 4 and 5, results revealed that most students had a positive opinion in all three blocks of questions (**Figure 7B** and **Supplementary Material**). In the block on scientific interest, 82.3% of the students, with 5 (33.3%) and 4 (49%), considered that their participation in the project sparked their interest in science, 75%, with 5 (37.7%) and 4 (37.3%), that it brought them closer to a real problem and 67.8%, with 5 (31%) and 4 (36.8%), that the experience had an impact on their knowledge of microbial diversity in the environment. However, only 45.4% of the students expressed that their results can contribute to scientific progress (rating 4–5 points). Regarding to antibiotic resistance, 76.7% of the students, with 5 (41.2%) and 4 (35.5%), also expressed that their participation in the project contributed to a better understanding of the problem of antibiotic resistance and 82.3%, with 5 (50.6%) and 4 (31.7%), that this experience modified their perception of the use of antibiotics. In the third block of the questionnaire, considering together scores of 4 and 5, 74.2% of the students answered that the project improved their scientific training and 87.8% showed a very positive global opinion about their participation in the project. Remarkably, 94% of students, with 5 (75.8%) and 4 (18.2%), would recommend to other colleagues or centers the participation in this initiative. In the block of free response, more than 50% of the students commented that one aspect that could be improved would be to substitute the use of disposable plastic gowns, about 40% of them indicated that the sessions should have lasted a longer time, and about a quarter of them expressed that the worst part of the project was not obtaining positive antibiosis results. However, the results of the surveys reflected the didactic value of the sessions since the students highly valued the acquisition of the concepts related to them, as well as their participation in a scientific research process.

In addition to the social objective of this SL project, another one was learning and curricular complementation within the biology subjects, in which there is no specific microbiology block. However, the practical sessions are considered transferable skills, in that students put into practice and acquire several key competences. High/secondary school students developed mathematical competence, since part of the results obtained during the isolation of soil bacteria have to be expressed through mathematical calculations. In addition to the basic competences of the project, the participants acquired social and civic ones, making them aware of the public health problem posed by antibiotic resistance and, as a consequence, turning them into informed and critical citizens.

Moreover, in the context of the *Natura* project, secondary school students also developed digital competence since they had to use computer applications to transmit the project contents to primary students. As well as the competence of language communication to transmit the results through different channels using a specific language. Additionally, they carried out the competence of initiative and entrepreneurial spirit since they were the ones who chose the way of preparing and transmitting the information to students of lower educational levels.

Regarding surveys carried out by secondary school teachers to evaluate the impact of the project on their students, results were also very satisfactory. Teachers highly valued the complementation of the students' practical skills. They also agreed on the suitability of these type of projects as a way to increase their students' interest in science (data not shown).

Overall, survey results for university and high/secondary school students were very positive and similar to those of Valderrama et al. (2018) and de Groot et al. (2020).

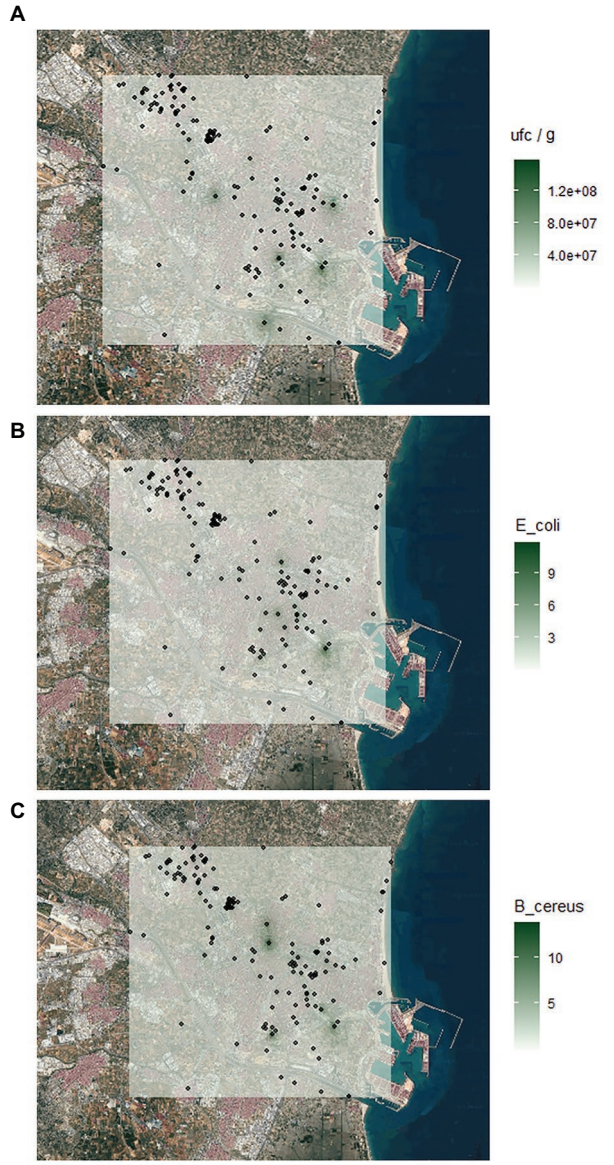

FIGURE 6 | Kriging interpolation of the number of **(A)** colony forming units per gram (CFU/g) of soil, **(B)** soil isolates inhibiting *Escherichia coli*, and **(C)** soil isolates inhibiting *Bacillus cereus* in the metropolitan area of Valencia.

Evaluation of Activities in Primary Schools

Results of evaluation surveys filled by primary school students participating in the *Natura* project in the academic course 2018–2019 are shown in **Figure 7C** and in **Supplementary Material**. The evaluation of the youngest students of the project was also very positive based on their answers to the six questions of the survey, reaching 5 points with percentages of 69–95% in five of them, on the scale of 1–5. Based on the responses to the first three questions related to their learning through the project activities "I have understood when or not I have to take antibiotics," "I have learned new things about bacteria and antibiotics," and "I think I know more things than before participating in the project" with the highest score of 5 with percentages of 95.2, 71.4, and 69%, respectively, the elementary students gave a very high valuation to the project. Likewise, in the following two questions about students' interest in the project activities and how this initiative has helped them become more interested in science, results revealed their high degree of satisfaction for 95.2 and 81% of them [rating 4 (14.2 and 38.1%) to 5 (81 and 42.9%) points in the two questions], respectively. Concerning the last question on whether the monitors did their job well, 95.2% of the primary school students rated it with 5 score.

Regarding the block of free response, about 85% of the students considered that they would not change anything in the activity, as well as that they really liked the theoretical and practical explanations. As negative aspects, a few students commented that they did not like the smell of bacterial cultures or having to wear a gown and gloves or considered that the sessions were too short. But, in general, primary students participating in the *Natura* project gave a very positive assessment of the scientific nature of the activity, as well as the acquisition of new knowledge and concepts related to the subject of natural sciences, which would reinforce the blocks of "Human beings and health" and that of "Living beings." Additionally, in the practical sessions carried out with the primary students, they were able to develop several key competences, scientific ones, since they participated in the execution of the practices and, not less important, social and civic ones, by making them aware of a health problem that affects the society as a whole.

Dissemination Activities

Different extramural dissemination actions were carried out over these 2 years within the *MicroMundo*@Valencia initiative. Firstly, around 500 children/teenagers, accompanied by their respective families or teachers, visited the *Expociencia* science fair and actively participated in the "antibiotics" workshop every year. They assayed an approach to the first step in the isolation of antibiosis producer strains whose results could be seen later by connecting to internet. In addition, hundreds of adults received theoretical explanations with the help of panels in order to raise their awareness on the rational and responsible use of antibiotics. Moreover, the adults were provided with an information card of the project, which encouraged several schools to contact us in order to join our initiative.

Secondly, we ran a novel extramural activity, an exhibition performed under the coverage of the specific subproject "DivulSuperBugs" (DSB). Thus, 14 infographics on the antibiotic resistance crisis, prepared by 42 students from the Biology degree at the UV, composed the exhibition which was coordinated by two or three university monitors in 14 secondary schools. The school students (average of 40 by center) visited it over a week and the monitors extended their involvement to a final session of direct learning using classroom quizzes. University students strongly engaged the younger students using the game-based learning platform Kahoot and escape rooms designed by themselves. In general, the school students highly valued the contribution of the exhibition to their understanding of bacterial resistance to antibiotics as well to become aware of their responsible use (data not shown).

FIGURE 7 | Two-year evaluation results of *MicroMundo*@Valencia activities by participating students in the Valencian Community. Anonymous surveys were carried out in the academic years 2017–2018 and 2018–2019, but the results are shown globally. The survey questionnaires were adapted according to the participation and educational level of the students. Response options were based on a 5-value Likert scale. **(A)** Surveys for *MicroMundo* Teaching Assistants (MMTAs; n = 140) consisted of 15 questions in four blocks in which they were asked about their opinion on the scientific interest of the project, their concept about antibiotic resistance, their experience as teachers for younger school students, and on their collaboration in the project. **(B)** Questionnaires for high/secondary school students (n = 628) included nine questions divided into three blocks to ask them about their scientific interest, their previous knowledge about antibiotic resistance, and their participation in the project. **(C)** Surveys for primary school students (n = 42) only included six questions about their learning experience, their interest in the project and their opinion on the high/secondary school students as monitors.

Extramural scientific activities were performed under the perspective of gender equality. In fact, to strengthen this philosophy, university female students explained our project in around 30 high schools in the Valencian Community (**Figure 3C**). They highlighted their role as scientists under training and the promising professional perspectives for students of STEM degrees, trying to motivate secondary female students toward these university careers. In Europe, the demand for university degrees increases by 14% each year, however, only a very small percentage of students chooses to enroll in higher education linked to STEM (Rohaan et al., 2010; Education and Training Monitor EU analysis, 2019). The figures for women are even lower, anecdotal in some STEM degrees, due to a combination of social, cultural, and economic factors. That is why the general objective of the global STEM and Gender Advancement (SAGA) projects of UNESCO is to contribute

to reduce the gender imbalance in STEM fields in all countries at all levels of education and research.[4]

As an important complement to inform a mainly young audience, we held over 40 conferences or round tables in regional high/secondary schools and contributed to the training of their science teachers (**Figure 3D**). We also participated in monographic conferences in Science Weeks running in towns of our Community (Gandia, Quart, …) and in singular scientific centers (Institute of Biomedicine of Valencia, City of Arts and Sciences of Valencia and Planetarium of Castellón).

In addition, within an academic context, several MMPIs and MMTAs attended the first SL *MicroMundo*@Valencia symposium held at the UCM in Madrid on July 2018, by presenting four oral communications and eight posters with the results of the project. Besides, our group also presented eight posters based on this SL experience on antibiotic resistance awareness in the IV Congress of the D+DM group of the SEM also held at the UCM. Two works presented by two MMPIs from the UV received awards, for the best poster and an *honorable mention*, respectively. Moreover, two MMTAs from BACPLANT group from UV, students of the master's degree in Molecular, Cellular, and Genetics Biology, also received the award for the winning video of the competition "Do something about antibiotic challenge." SWI/CDC/NIH/SEM (Link: https://youtu.be/q92S574-NoM). Throughout these years, we have collaborated with broadcasting companies (**Supplementary Table S1**), the most relevant contributions being two documentaries on the public television channel *À punt*,[5] several radio programs,[6] and a mini report in the newspaper with the largest circulation in our region.[7] Moreover, numerous references have appeared in the local written press and radio stations. These diverse actions amply demonstrate the recognition achieved by the project in the academic context, as well as significant media coverage.

CONCLUSION

The implementation of *MicroMundo*@Valencia initiative at the Universitat de València has increased the awareness of the public health problem posed by microbial antibiotic resistance in university, high/secondary and primary school teachers and students participating in the project in the Valencian Community, and has made possible their involvement in the active search for solutions. As a result, over 7,000 bacterial isolates were obtained from more than 300 soil samples, increasing the chances of finding new antimicrobials. In fact, some of these environmental isolates were able to inhibit the growth of target bacteria and could be tested against diverse pathogens in the future. In addition, geomapping of sampling sites combined with Kriging analysis allowed the localization of soil foci of antibiotic-producing bacteria, suggesting the potential of the application developed to optimize antimicrobial research across large geographical regions.

Evaluation of the project, based on student surveys, revealed that most of them acquired the expected scientific and pedagogical concepts and skills, improving their self-learning capacity and their academic and social engagement. This confirmed SL as a powerful teaching strategy that, in turn, allowed students involved to become a "transmission belt" to spread awareness of the problem of antibiotic abuse to society. Moreover, the project was able to promote student interest in science through the discovery of soil bacteria producing antimicrobials as well as to enable mutual cooperation among students of three different education levels. Further, the implementation of this initiative in a primary school, as a pilot experience, allowed the secondary and primary students involved to develop civic, social, and scientific competences, among others. Finally, *MicroMundo*@Valencia was also extended through additional outreach activities outside the university, and this generated sensitivity in the Valencian Community toward the global antibiotic crisis, while raising awareness of it.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, and further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors participated in the implementation of *MicroMundo*@Valencia at the UV and in high/secondary schools, and SM, ÀF-S, and EB also in a primary school. JS-G developed the APP for geolocate sampling sites. EC determined properties of soil samples. SM, BF, ÀF-S, HR, and EB participated in extramural activities. SM, BF, and EB conceived and designed the manuscript. All authors contributed to the manuscript by drafting the work or revising it. SM, BF, ÀF-S, HR, JS-G, and EB generated figures and/or tables. SM, ÀF-S, EC, JS-G, and EB analyzed the data. All authors approved the final version of the manuscript.

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[4]http://www.unesco.org/new/en/natural-sciences/priority-areas/gender-and-science/improving-measurement-of-gender-equality-in-stem/

[5]https://apuntmedia.es/va/noticies/punt-docs/21-12-2018-l-amenaca-dels-bastonets and https://vimeo.com/388287770

[6]https://vimeo.com/388287770 and https://www.ivoox.com/a-ciencia-cierta-18-11-2019-la-verdad-sobre-los-audios-mp3_rf_44430590_1.html

[7]https://www.levante-emv.com/aula/2018/02/21/joves-recerca-nous-antibiotics/1681964.html

(SFPIE_RMD17_588566, SFPIE_GER17-588199, SFPIE_RMD18_839102, SFPIE_GER18-844761, SFPIE_RMD18_954062, SFPIE_PID19-1096784, and SFPIE_PID19_1096021).

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.564030/full#supplementary-material

REFERENCES

Abraham, E. P., and Chain, E. (1940). An enzyme from bacteria able to destroy penicillin. *Nature* 146:837. doi: 10.1038/146837a0

Abrahamsen, L. (2004). Learning partnerships between undergraduate biology students and younger learners. *Microbiol. Educ.* 5, 21–29. doi: 10.1128/jmbe.v5.74

Burnham, J. P., Olsen, M. A., and Kollef, M. H. (2019). Re-estimating annual deaths due to multidrug-resistant organism infections. *Infect. Control Hosp. Epidemiol.* 40, 112–113. doi: 10.1017/ice.2018.304

Caruso, J. P., Israel, N., Rowland, K., Lovelace, M. J., and Saunders, M. J. (2016). Citizen science: the Small World Initiative improved lecture grades and California critical thinking skills test scores of nonscience major students at Florida Atlantic University. *J. Microbiol. Biol. Educ.* 17, 156–162. doi: 10.1128/jmbe.v17i1.1011

Celio, C. I., Durlak, J., and Dymnicki, A. (2011). A meta-analysis of the impact of service-learning on students. *J. Exp. Educ.* 34, 164–181. doi: 10.1177/105382591103400205

Davis, E., Sloan, T., Aurelius, K., Barbour, A., Bodey, E., Clark, B., et al. (2017). Antibiotic discovery throughout the Small World Initiative: a molecular strategy to identify biosynthetic gene clusters involved in antagonistic activity. *MicrobiologyOpen* 6:e435. doi: 10.1002/mbo3.435

de Groot, P. W. J., Fernández-Pereira, J., Sabariegos, R., Clemente-Casares, P., Parra-Martínez, J., Cid, V. J., et al. (2020). Optimizing Small World Initiative service learning by focusing on antibiotics-producing actinomycetes from soil. *FEMS Microbiol. Lett.* 366:fnaa019. doi: 10.1093/femsle/fnaa019

Education and Training Monitor EU analysis (2019). https://ec.europa.eu/education/sites/education/files/document-library-docs/volume-1-2019-education-and-training-monitor.pdf (Accessed April 24, 2020).

Emmert, E. A. (2013). Biosafety guidelines for handling microorganisms in the teaching laboratory: development and rationale. *J. Microbiol. Biol. Educ.* 14, 78–83. doi: 10.1128/jmbe.v14i1.531

Esson, J. M., and Stevens-Truss, R. (2005). Service-learning in introductory chemistry: supplementing chemistry curriculum in elementary schools. *J. Chem. Educ.* 82, 1168–1173. doi: 10.1021/ed082p1168

Hernandez, S., Tsang, T., and Handelsman, J. (2015). *Small World Initiative: Research protocols*. Connecticut: Yale University.

Hibbing, M. E., Fuqua, C., Parsek, M. R., and Peterson, S. B. (2010). Bacterial competition: surviving and thriving in the microbial jungle. *Nat. Rev. Microbiol.* 8, 15–25. doi: 10.1038/nrmicro2259

Joseph, S. J., Hugenholtz, P., Sangwan, P., Osborne, C. A., and Janssen, P. H. (2003). Laboratory cultivation of widespread and previously uncultured soil bacteria. *Appl. Environ. Microbiol.* 69, 7210–7215. doi: 10.1128/AEM.69.12.7210-7215.2003

Lies, J. M., Bock, T., Brandenberger, J., and Trozzolo, T. A. (2012). The effects of off-campus service learning on the moral reasoning of college students. *J. Moral Educ.* 41, 189–199. doi: 10.1080/03057240.2012.669925

Likert, R. (1932). A technique for the measurement of attitudes. *Arch. Psychol.* 140, 1–55.

Mansour, A., Zeinab, R., and Amanollah, Z. A. (2015). Isolation and identification of *Bacillus* species from soil and evaluation of their antibacterial properties. *Avicenna J. Clin. Microbiol. Infect.* 2, 1–4. doi: 10.17795/ajcmi-23233

MAPA (1994). Official methods for soil and water analysis. *Vol. 3*. Madrid, Spain: Ministry of Agriculture, Fisheries and Food.

McCaig, A. E., Grayston, S. J., Prosser, J. I., and Glover, L. A. (2001). Impact of cultivation on characterisation of species composition of soil bacterial communities. *FEMS Microbiol. Ecol.* 35, 37–48. doi: 10.1111/j.1574-6941.2001.tb00786.x

Miller, S. I. (2016). Antibiotic resistance and regulation of the Gram-negative bacterial outer membrane barrier by host innate immune molecules. *mBio* 7, e01541–e01516. doi: 10.1128/mBio.01541-16

Mulani, M. S., Kamble, E. E., Kumkar, S. N., Tawre, M. S., and Pardesi, K. R. (2019). Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. *Front. Microbiol.* 10:539. doi: 10.3389/fmicb.2019.00539

Navidinia, M. (2016). The clinical importance of emerging ESKAPE pathogens in nosocomial infections. *J. Paramed. Sci.* 7, 2008–4978. doi: 10.22037/jps.v7i3.12584

Rammelkamp, C. H., and Maxon, T. (1942). Resistance of *Staphylococcus aureus* to the action of penicillin. *Proc. Soc. Exp. Biol. Med.* 51, 386–389. doi: 10.3181/00379727-51-13986

Rice, L. B. (2008). Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J. Infect. Dis.* 197, 1079–1088. doi: 10.1086/533452

Rohaan, E. J., Taconis, R., and Jochems, W. (2010). Reviewing the relations between teachers' knowledge and pupils' attitude in the field of primary technology education. *Int. J. Technol. Des. Educ.* 20, 15–26. doi: 10.1007/s10798-008-9055-7

Sessitsch, A., Weilharter, A., Gerzabek, M. H., Kirchmann, H., and Kandeler, E. (2001). Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. *Appl. Environ. Microbiol.* 67, 4215–4224. doi: 10.1128/AEM.67.9.4215-4224.2001

Silver, W. L., Neff, J., McGroddy, M., Veldkamp, E., Keller, M., and Cosme, R. (2000). Effects of soil texture on belowground carbon and nutrient storage in a lowland Amazonian forest ecosystem. *Ecosystems* 3, 193–209. doi: 10.1007/s100210000019

Stubbendieck, R. M., and Straight, P. D. (2016). Multifaceted interfaces of bacterial competition. *J. Bacteriol.* 198, 2145–2155. doi: 10.1128/JB.00275-16

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., et al. (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect. Dis.* 18, 318–327. doi: 10.1016/S1473-3099(17)30753-3

Valderrama, M. J., González-Zorn, B., de Pablo, P. C., Díez-Orejas, R., Fernández-Acero, T., Gil-Serna, J., et al. (2018). Educating in antimicrobial resistance awareness: adaptation of the Small World Initiative program to service-learning. *FEMS Microbiol. Lett.* 365:fny161. doi: 10.1093/femsle/fny161

Voroney, R. P. (2007). "The soil habitat" in *Soil microbiology, ecology and biochemistry*. ed. E. A. Paul (London: Elsevier, Academic Press), 25–49.

Wackernagel, H. (2003). *Multivariate geostatistics: An introduction with applications*. *3rd Edn.* Berlin, Germany: Springer.

Wang, C. -H., Hsieh, Y. -H., Powers, Z. M., and Kao, C. -Y. (2020). Defeating antibiotic-resistant bacteria: exploring alternative therapies for a post-antibiotic era. *Int. J. Mol. Sci.* 21:1061. doi: 10.3390/ijms21031061

Webb, G. (2017). A review of microbiology service learning. *FEMS Microbiol. Lett.* 364:fnx032. doi: 10.1093/femsle/fnx032

Zhang, H., Voroney, R. P., and Price, G. W. (2014). Effects of biochar amendments on soil microbial biomass and activity. *J. Environ. Qual.* 43, 2104–2114. doi: 10.2134/jeq2014.03.0132

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Using Metabolic Engineering to Connect Molecular Biology Techniques to Societal Challenges

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Genetically modified organisms (GMOs) are a topic of broad interest and are discussed in classes ranging from introductory biology to bioethics to more advanced methods-focused molecular biology courses. In most cases, GMOs are discussed in the context of introducing a single protein-coding gene to produce a single desired trait in a crop. For example, a commercially available kit allows students to test whether food products contain GMOs by detecting the *Bacillus thuringiensis* delta-endotoxin gene, which confers resistance to European corn borers. We have developed an 8-week laboratory module for upper-division undergraduates and graduate students that builds upon students' basic understanding of GMOs to introduce them to the techniques used to sustainably produce commercially valuable products in yeast through metabolic engineering. In this course, students use recombination-based methods to assemble genes encoding entire metabolic pathways in *Saccharomyces cerevisiae*, perform genetic screens to identify yeast genes that impact metabolite yield, and use error-prone PCR to optimize metabolic pathway function. In parallel to these laboratory-based activities, students engage with the societal impact of these approaches through case studies of products made via yeast metabolic engineering, such as opioids, omega-3 fatty acids, and the Impossible Burger. In this report, we focus on these case studies as well as an individual sustainability project assignment created for this course. This assignment, which spans the 8-week module, asks students to find examples of yeast metabolic engineering that could be used to address current sustainability challenges in their communities. By the end of the course, students synthesize this information to create a case study that could be used to teach concepts related to metabolic engineering and sustainability to their peers. Student approaches to this project have varied from literature reviews, to news searches, to directly contacting and interviewing researchers using novel metabolic engineering approaches. These student-produced projects are used as case studies in future semesters, amplifying student voices and contributing to student ownership. While developed in the context of this course, the sustainability project and case studies are broadly applicable and could be adapted for use in biology or bioethics courses at the undergraduate or graduate level. Through this report, we hope to gain collaborators interested in implementing a version of the course at their institutions, allowing for robust assessment of the impact of the course on a larger group of students.

Keywords: metabolic engineering, yeast, sustainability, case study, molecular biology, group work, agency, ownership

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INTRODUCTION

As arguably one of the best-characterized, safest, least expensive, and most genetically tractable model organisms, *Saccharomyces cerevisiae* is an appealing choice for use in teaching labs (Sitaraman, 2011; Wolyniak, 2013; Bowling et al., 2016; Oelkers, 2017; Hageman and Krikken, 2018; Pedwell et al., 2018; Sehgal et al., 2018; de Waal et al., 2019). Yeast has been used as a model system for a variety of different courses and educational activities. Students have worked with *S. cerevisiae* to learn about Mendelian genetics and molecular biology concepts (Wolyniak, 2013), deletion of genes in yeast (Hageman and Krikken, 2018), transcriptional regulation (Oelkers, 2017), and the creation of frameshifts using CRISPR/Cas9 (de Waal et al., 2019). Several published course-based undergraduate research experiences (CUREs) allow students to contribute novel findings while learning fundamental molecular biology and genetics skills (Bowling et al., 2016; Pedwell et al., 2018). Wild yeasts have also been used to engage the public as citizen scientists with at-home experiments (Nichols, n.d.). The availability of curated databases (e.g., Saccharomyces Genome Database SGD) (Saccharomyces Genome Database [SGD], n.d a), genetic tools, and adaptable protocols make *S. cerevisiae* an attractive and powerful model system for lab-based courses that can transform our students as scientists.

In redesigning an existing yeast genetics course, our dual goals were to implement the elements of a course-based research experience (CRE) and to engage students in thinking beyond the science to its social implications. As we began this redesign, we were inspired by Jef Boeke's lab's work developing synthetic biology tools in yeast. In the redesigned course, we aimed to use the Yeast Golden Gate (yGG) and the versatile genetic assembly system (VEGAS) methods created by the Boeke lab (Agmon et al., 2015; Mitchell et al., 2015) to engage students in metabolic engineering of yeast to produce the red-orange vitamin A precursor beta-carotene, which is used in foods, feeds, cosmetics, and pharmaceuticals (Nelis and Leenheer, 1991; Anunciato and da Racho Filho, 2012; Li et al., 2013). The lab component of the course emphasized applications of yeast metabolic engineering, while the lecture focused on sustainability and the societal implications of the use of engineered yeast as "sustainable" sources of commercial products. While a robust evaluation of the impact of the course redesign on student engagement will require assessment of multiple cohorts of students over several years, student participation in class discussions, informal conversations during lab sessions, and enthusiasm during end-of-semester poster presentations suggest that the CRE structure facilitated engagement.

COURSE DESCRIPTION

The Yeast Metabolic Engineering course is an 8-week long lab module enrolling juniors, seniors, and graduate students from across multiple majors, programs, and colleges at NC State University in Raleigh, NC. It is structured as one 2-h lecture session and one 5-h lab session per week and housed within the Biotechnology Teaching Program (BIT). As a BIT course, it is designed to teach students cutting-edge technical skills that can be applied in academic research or in industry. The prerequisite for enrolling in lab modules is the completion of a semester-long lab-based molecular biology course (Carson et al., 2019; Garcia et al., 2020). As technology changes, BIT courses are regularly redesigned and updated to reflect current research and molecular biotechnology tools. As we worked to redesign this course, our two goals were: (1) Adopting elements of a course-based research experience (CRE) in the lab, and (2) Engaging students in thoughtful discussion of the societal implications of yeast metabolic engineering, with a focus on sustainability (**Figure 1A**).

The course was developed using backward design, with the following course goals:

1. **Design** and **troubleshoot** experiments to grow and genetically manipulate *S. cerevisiae*.
    a. **Verify** the genotype of yeast strains.
    b. **Engineer** constructs for overproduction of beta-carotene.
    c. **Transform** yeast with recombinant DNA constructs.
    d. **Assess** the viability of genetically engineered yeast.
    e. **Measure** the intensity of pigmentation (beta-carotene) in different strains.
2. **Interpret** data associated with metabolic engineering of yeast.
3. **Identify** limitations and alternative approaches associated with the metabolic engineering of yeast.

Weekly lecture and lab sessions had assignments with objectives designed to align with these course goals. Assessments included quizzes, case studies, reading assignments, electronic lab notebook entries, lab reports, posters, a final exam, and a sustainability project for graduate students.

LAB COMPONENT: SCIENTIFIC PRACTICE, COLLABORATION, DISCOVERY, AND ITERATION

Of the 8 weeks in this course, students spend 6 weeks working to optimize the production of a commercially useful metabolite in *S. cerevisiae*. In the current iteration of this course, students produce beta-carotene, a vitamin A precursor. This particular product was selected because students can easily perform initial phenotypic screens, as beta-carotene-producing colonies are visibly orange. Production of beta-carotene requires the introduction of three exogenous genes encoding enzymes that catalyze the stepwise conversion of acetyl coA to beta-carotene, and optimal production is obtained when the gene encoding a truncated form of Hmg1, an endogenous *S. cerevisiae* protein, is overexpressed (Verwaal et al., 2007; Li et al., 2013; Mitchell et al., 2015). To achieve this, students assemble these four genes, along with a KanR selection cassette, using the versatile genetic assembly system [VEGAS; (Mitchell et al., 2015)]. VEGAS exploits the capacity of *S. cerevisiae* to join sequences with terminal homology by homologous recombination: students transform yeast with a digested acceptor plasmid and individual

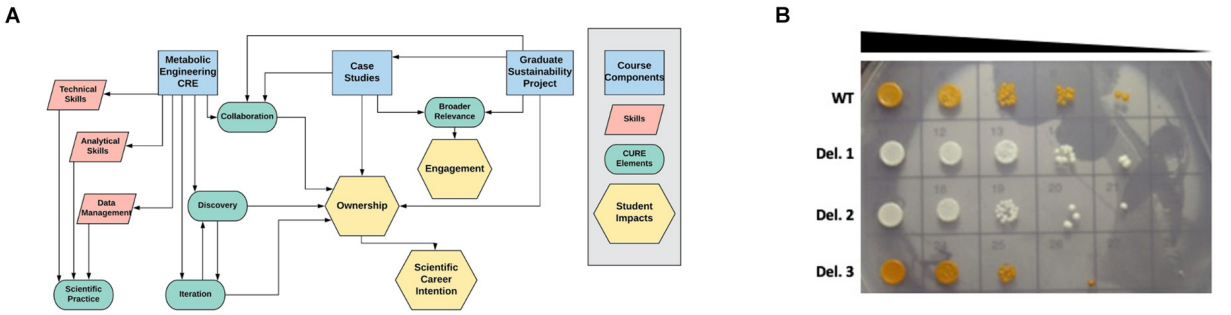

FIGURE 1 | Course design and example student data. **(A)** The three main components of the Yeast Metabolic Engineering course (blue rectangles) were designed to train students in specific skills (pink parallelograms) while incorporating the five elements of course-based undergraduate research experiences (green ovals), leading to the student impacts of engagement, ownership, and scientific career intention (yellow hexagons). **(B)** Students performed VEGAS to assemble the beta-carotene biosynthetic pathway in wild type (WT) yeast or a pool of barcoded single gene deletion strains. Transformants displaying decreased beta-carotene production (white colonies) or increased beta-carotene production (dark orange colonies) were selected for spot plate analysis to compare genetic fitness and barcode sequencing to identify the deleted genes. In some cases, these experiments resulted in novel findings. Deletion strain 3 (Del. 3) in this student experiment displayed increased accumulation of beta-carotene, and barcode sequencing identified this strain as an Mcr1 deletion mutant. This finding suggests that interfering with competing pathways, such as the ergosterol biosynthetic pathway, can drive increased beta-carotene accumulation.

transcriptional units (TUs) consisting of a promoter, gene of interest, and terminator, each flanked with orthogonal adapter sequences (Agmon et al., 2015). These adapter sequences are designed with terminal homology such that homologous recombination assembles the genes in the desired order into the plasmid (Mitchell et al., 2015).

To increase student engagement and ownership of the lab-based project, we have incorporated four elements of course-based research experiences supported by evidence as best practices: scientific practice, collaboration, discovery, and iteration (Auchincloss et al., 2014; Brownell and Kloser, 2015; Corwin et al., 2018; **Figure 1A**). Importantly, collaboration, discovery, and iteration have been shown to increase not only student ownership but also students' intention to pursue a scientific career (Corwin et al., 2018).

Collaboration is achieved through both the laboratory and lecture portions of the course. In the lab, students work in pairs to carry out research projects that contribute to the overarching goal of identifying genetic changes that improve beta-carotene yield. Thus, each student collaborates with their partner, while the pairs collaborate as one larger research group. In the lecture portion of the class, students can choose to work in groups on in-class activities, case studies, and the graduate sustainability project. The groups students form for these activities and assignments tend to include 3–4 students, allowing for collaboration within larger groups than in the lab. In contrast to most CUREs, our course enrolls both undergraduates and graduate students. This allows for collaboration among students at different academic levels and the formation of "near-peer" mentoring relationships, which have been shown to benefit both the mentor and the mentee (Tenenbaum et al., 2014; Trujillo et al., 2015).

While the lab portion of the class utilizes published techniques for assembling a well-characterized metabolic pathway, the element of discovery is added by performing two different genetic screens to further optimize metabolite production. This approach differs from other courses in which students produce beta-carotene in yeast, such as the Cold Spring Harbor Laboratory Yeast Genetics and Genomics course (Dunham et al., 2015), which focus on techniques rather than experimental design and inquiry.

Our students engage in discovery through two different genetic screening experiments: (1) Assembly of the beta-carotene pathway in a pool of barcoded yeast gene deletion strains (Winzeler et al., 1999; Giaever et al., 2002), followed by barcode sequencing of beta-carotene over- or under-producers to identify novel genes involved in beta-carotene production; and (2) Error-prone PCR amplification of TUs to optimize beta-carotene production through random mutagenesis of the genes encoding the metabolic pathway. Each pair of students selects 2–3 strains from each of these experiments for sequencing analysis based on visual assessment of beta-carotene production, resulting in a total of approximately 15–20 strains analyzed per semester. While the throughput of this genetic screen could be increased, allowing each group to select and work with their own strains results in a sense of ownership, as groups compete to see which strain will produce the highest beta-carotene yield. Moreover, even with this simple, low-throughput genetic screen, student projects in this course have resulted in novel findings. For example, the deletion of the gene Mcr1, which encodes a mitochondrial NADH-cytochrome b5 reductase that is involved in ergosterol biosynthesis (Hahne et al., 1994; Lamb et al., 1999), results in increased production of beta-carotene (**Figure 1B** and M. Calzini and M. Whittaker, unpublished data). Discovering new phenotypes and identifying novel roles for genes in the production of beta-carotene encourages students to investigate the literature and to design future experiments to further enhance desired phenotypes. In this way, these experiments model an authentic scientific process and facilitate student agency (Hester et al., 2018).

Importantly, sufficient time is built into the lab schedule to allow groups to repeat experiments when necessary, rather

than providing students with "back-up" PCR products or transformants. This iteration further promotes ownership, as students carry out every step of their experiment, and have the opportunity to learn from mistakes and master new technical skills.

LECTURE COMPONENT: ENGAGEMENT WITH SOCIAL ISSUES

Engagement With Social Issues: Case Studies

The lecture portion of the course focuses on applications and societal implications of yeast metabolic engineering to achieve the fifth element of CUREs: broader relevance (Auchincloss et al., 2014; Brownell and Kloser, 2015). Student engagement with these topics is achieved through the use of three case studies. Cases have been shown to provide realistic scenarios that require critical thinking within a structure that can be used to promote engagement, motivation, and information retention (Allchin, 2013). Cases provide *context* and can be used to connect real-world problems with the topics or technologies discussed in the course. More than teasers and "hooks," cases can be scheduled in a course to bring forth societal issues that complement lab techniques. We sequenced the cases we developed to intentionally introduce current applications and societal challenges addressed by the use of engineered yeast (**Table 1**). Moreover, a common thread was the emphasis on the sustainability of these approaches and their impact on *all* members of our society.

Cases were assigned as digital Google Docs worksheets via Doctopus (Wright et al., 2014), and students were allowed to work in groups or independently. Each case was introduced in class, and students were given approximately 30–45 min to begin working. Groups continued collaborating through Google Docs to finish the case studies outside of class. All case studies included learning outcomes, background information (often videos and related articles), and a series of questions for analysis. Questions required students to think critically about how yeast was used to produce the Impossible Burger (Case Study 1), opioids (Case Study 2), and omega-3 fatty acids (Case Study 3). Several questions then asked participants to compare the methods and approaches used in the different case studies. All cases included questions that prompted participants to reflect and think about: (a) whether this is an example of yeast metabolic engineering, (b) whether the approach is "sustainable," (c) why is yeast genetic engineering and/or metabolic engineering useful for the production of this substance, and (d) the ethical implications of the technology.

Groups received feedback in their Google Docs correcting any misconceptions and asking additional questions to push students to think more deeply about sustainability. Most students in this course have completed BIT 501: Ethical Issues in Biotechnology and have a basic understanding of bioethics and a framework for discussing the social implications of biotechnological innovations. Based on the previous coursework and academic background of our students, we expected students to focus largely on environmental sustainability in their group answers. During the class period following each case study, a class-level discussion was used to extend students' understanding beyond environmental sustainability to include economic and social sustainability. Typically, one or two groups had already considered these facets of sustainability in their Google Docs, and their comments during the class discussion sparked other groups to expand their initial treatment of the sustainability implications of the technologies discussed. Following the class discussion, instructors summarized key points in a course announcement. Expected answers to the questions posed in the Google Docs case studies as well as examples of topics typically addressed during class

TABLE 1 | Summary of case studies used to engage students in discussion of ethical and societal issues raised and addressed by advances in yeast metabolic engineering.

Case study and associated publications	Yeast species used	Metabolic engineering approach	Impact on sustainability	Ethical questions and societal issues
Impossible Burger (PLoS, 2019; Wired, n.d.; FAQ, n.d a)	*Pichia pastoris*	Introduction of a single gene encoding soy leghemoglobin	Creation of a meat-free burger; reduced emissions and water use	Can the Impossible Burger actually be considered vegan? Is the goal to produce a burger for vegetarians, or to convince meat-eaters to eat less meat?
Semisynthetic Opioid Production in Yeast (Galanie et al., 2015; Saccharomyces Genome Database [SGD], n.d b)	*S. cerevisiae*	Introduction of 16 genes from five different organisms to encode an entire metabolic pathway	Reduced water and fertilizer use compared to production from poppies	Potential for individuals to create illicit drugs. Yield of desired product is very low – is this method feasible?
Engineered Yeast and Verlasso Salmon (Xie et al., 2015; FAQ, n.d b)	*Yarrowia lipolytica*	Introduction of multiple pathways genes using marker recycling to encode the metabolic pathway needed to produce omega-3 and omega-6 fatty acids into *Y. lipolytica*, which was used in place of feeder fish to feed salmon	Reduced need for feeder fish for farmed salmon	Engineered yeast released into the ocean. After initial investment and work, DuPont no longer uses this strategy - why?

discussions (including all aspects of sustainability, as well as ethical and social justice considerations) are included in the Case Study Keys.

Engagement With Social Issues: Graduate Sustainability Project

In addition to case study-based learning, graduate students enrolled in the course were required to complete a 3- to 5-page report discussing the use of yeast metabolic engineering for sustainable production of food, resources, biofuels, or bioremediation, referring to at least three primary literature publications. In addition to explaining the techniques used to engineer yeast, optimize yield, and generate the product, students were asked to explore state, local, and, if applicable, campus-wide initiatives related to their project and to propose a future direction or application that relates to the current economic growth and needs of our state and campus.

This assignment further promotes engagement by allowing choice. Students typically choose to analyze the sustainable generation of a product related to their own research or personal interests. The ability to connect the project to existing interests and societal concerns helps students to contextualize the science and reflect on its implications. The format of the project and guidelines offer structure and intentionally include the evaluation of scholarly research (primary references), summaries of complex genetic engineering processes for a lay audience, and connections to local and national societal issues. The assignment includes at least one figure or table, and students often produce graphical abstracts.

Grading is based on a modification of specifications grading, with clear requirements for A, B, and C work. An opportunity to earn bonus points is included, and this is often met when students go beyond the stated elements of the project and include information about local/campus-wide initiatives, interviews, or research that is impactful. For example, one exemplary project discussed the production of pigments for solar panels using yeast metabolic engineering, an approach that is not yet developed commercially and is understudied but has tremendous potential. Another project included first-person interviews with the researchers working to develop a yeast metabolic engineering-based technology.

Importantly, topics addressed in this project often become starting points for discussions and assignments in future versions of the class. In this way, these student-driven projects form the basis for case studies used in subsequent semesters. For example, in Spring 2021, we will use a new case study comparing the production of pigments for solar panels to the production of pigments for textiles using yeast metabolic engineering. This new case study combines two previous graduate projects in a way that lets students explore the similarities and differences in methods, technical challenges, and societal implications. Although the evolution of graduate student projects into case studies for future iterations of the course necessarily occurs after the end of the semester, when students are no longer enrolled, students are invited to participate in case study development and offered co-authorship if the case study results in a publication.

DISCUSSION

This course provides an example of a course structure intentionally designed to increase ownership and agency through the incorporation of critical elements of CUREs while also engaging students through case studies and projects focused on connecting the science to societal issues. Participants have actively searched for local technologies, companies, and researchers using engineered yeast to continue discussions and propose future applications of yeast metabolic engineering. For the graduate sustainability project, students have interviewed researchers, found examples of these technologies being used on our campus, and connected these efforts to their own research.

As the class continues to evolve, we plan to work with students to extend discussions about yeast metabolic engineering and sustainability beyond the classroom through the creation of podcasts, a website, and other public-facing educational materials. We also plan to strengthen the connection between this course and the existing Ethical Issues in Biotechnology course by inviting instructors and students in that course to provide feedback to Yeast Metabolic Engineering students on their case studies and graduate projects. Furthermore, discoveries made in the context of this course will be used as starting points for future undergraduate independent research projects and course offerings. For example, libraries of mutants produced by the class can be further analyzed by students in a course focused on high-throughput technologies and automation (e.g., BIT 479/579 *High-throughput Discovery*). Future educational studies will assess the impact of these course structures and practices on learning gains, engagement in sustainability efforts in and out of class, and student agency.

The equipment needed to offer this course – shaking and stationary incubators, spectrophotometers, centrifuges, themocyclers, and gel electrophoresis equipment – is often accessible to teaching labs. Reagents are either commercially available (e.g., Zymo Research, TransOMIC) or can be obtained from academic labs. The full electronic lab manual for this course is available from the authors upon request.

Students are genuinely excited by the ability to engineer yeast and produce a commercially relevant product that can be readily connected to their lived experience. The striking phenotype of yeast producing beta-carotene combined with the number of mutant libraries and genetic screens that can be adapted make this lab module an option for numerous different courses, student levels, and institutions. The VEGAS approach can be used to assemble other genetic pathways of interest that can be selected for and linked to societal, environmental, and public health needs. Importantly, the skills gained in the lab working with the model organism combined with ethical and social justice discussions directly linked to the applications of these technologies reinforce the professional development skills required of modern-day molecular biologists.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

CLG and CCG designed the course, adapted the lab experiments for teaching labs and course learning objectives, designed the case study and graduate project assignments, and wrote the manuscript. Both authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.577004/full#supplementary-material

REFERENCES

Agmon, N., Mitchell, L. A., Cai, Y., Ikushima, S., Chuang, J., Zheng, A., et al. (2015). Yeast Golden Gate (yGG) for the efficient assembly of *S. cerevisiae* transcription units. *ACS Synth. Biol.* 4, 853–859. doi: 10.1021/sb500372z

Allchin, D. (2013). Problem- and case-based learning in science: an introduction to distinctions, values, and outcomes. *CBE Life Sci. Educ.* 12, 364–372. doi: 10.1187/cbe.12-11-0190

Anunciato, T. P., and da Racho Filho, P. A. (2012). Carotenoids and polyphenols in nutricosmetics, nutraceuticals, and cosmeceuticals. *J. Cosmet. Dermatol.* 11, 51–54. doi: 10.1111/j.1473-2165.2011.00600.x

Auchincloss, L. C., Laursen, S. L., Branchaw, J. L., Eagan, K., Graham, M., Hanauer, D. I., et al. (2014). Assessment of course-based undergraduate research experiences: a meeting report. *CBE Life Sci. Educ.* 13, 29–40. doi: 10.1187/cbe.14-01-0004

Bowling, B. V., Schultheis, P. J., and Strome, E. D. (2016). Implementation and assessment of a yeast orphan gene research project; involving undergraduates in authentic research experiences and progressing our understanding of uncharacterized open reading frames. *Yeast Chichest. Engl.* 33, 43–53. doi: 10.1002/yea.3139

Brownell, S. E., and Kloser, M. J. (2015). Toward a conceptual framework for measuring the effectiveness of course-based undergraduate research experiences in undergraduate biology. *Stud. High. Educ.* 40, 525–544. doi: 10.1080/03075079.2015.1004234

Carson, S., Miller, H., Srougi, M., and Witherow, D. S. (2019). *Molecular Biology Techniques: A Classroom Laboratory Manual*. Amsterdam: Elsevier.

Corwin, L. A., Runyon, C. R., Ghanem, E., Sandy, M., Clark, G., Palmer, G. C., et al. (2018). Effects of discovery, iteration, and collaboration in laboratory courses on undergraduates' research career intentions fully mediated by student ownership. *CBE Life Sci. Educ.* 17:141. doi: 10.1187/cbe.17-07-0141

de Waal, E., Tran, T., Abbondanza, D., Dey, A., and Peterson, C. (2019). An undergraduate laboratory module that uses the CRISPR/Cas9 system to generate frameshift mutations in yeast. *Biochem. Mol. Biol. Educ.* 47, 573–580. doi: 10.1002/bmb.21280

Dunham, M., Gartenberg, M., and Brown, G. (2015). *Methods in Yeast Genetics and Genomics, 2015 Edn, A CSHL Course Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

FAQ (n.d a). *What are the Ingredients?*. Available online at: http://faq.impossiblefoods.com/hc/en-us/articles/360018937494 (accessed June 27, 2020).

FAQ (n.d b). Available online at: https://www.verlasso.com/faq (accessed June 27, 2020).

Galanie, S., Thodey, K., Trenchard, I. J., Filsinger Interrante, M., and Smolke, C. D. (2015). Complete biosynthesis of opioids in yeast. *Science* 349, 1095–1100. doi: 10.1126/science.aac9373

Garcia, C. B., Chapman, I. F., Chen, S. H., Lazear, E., Lentz, T. B., Williams, C., et al. (2020). Integrating research into a molecular cloning course to address the evolving biotechnology landscape. *Biochem. Mol. Biol. Educ.* 22, 1249–1252. doi: 10.1002/bmb.21402

Giaever, G., Chu, A. M., Ni, L., Connelly, C., Riles, L., Véronneau, S., et al. (2002). Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* 418, 387–391. doi: 10.1038/nature00935

Hageman, J., and Krikken, A. M. (2018). Single-Step Gene Knockout of the SUC2 Gene in *Saccharomyces cerevisiae*?: a laboratory exercise for undergraduate Students †. *J. Microbiol. Biol. Educ.* 19:19.3.92. doi: 10.1128/jmbe.v19i3.1615

Hahne, K., Haucke, V., Ramage, L., and Schatz, G. (1994). Incomplete arrest in the outer membrane sorts NADH-cytochrome b5 reductase to two different submitochondrial compartments. *Cell* 79, 829–839. doi: 10.1016/0092-8674(94)90072-8

Hester, S. D., Nadler, M., Katcher, J., Elfring, L. K., Dykstra, E., Rezende, L. F., et al. (2018). Authentic inquiry through modeling in biology (AIM-Bio): an introductory laboratory curriculum that increases undergraduates' scientific agency and skills. *CBE Life Sci. Educ.* 17:90. doi: 10.1187/cbe.18-06-0090

Lamb, D. C., Kelly, D. E., Manning, N. J., Kaderbhai, M. A., and Kelly, S. L. (1999). Biodiversity of the P450 catalytic cycle: yeast cytochrome b5/NADH cytochrome b5 reductase complex efficiently drives the entire sterol 14-demethylation (CYP51) reaction. *FEBS Lett.* 462, 283–288. doi: 10.1016/s0014-5793(99)01548-3

Li, Q., Sun, Z., Li, J., and Zhang, Y. (2013). Enhancing beta-carotene production in *Saccharomyces cerevisiae* by metabolic engineering. *FEMS Microbiol. Lett.* 345, 94–101. doi: 10.1111/1574-6968.12187

Mitchell, L. A., Chuang, J., Agmon, N., Khunsriraksakul, C., Phillips, N. A., Cai, Y., et al. (2015). Versatile genetic assembly system (VEGAS) to assemble pathways for expression in *S. cerevisiae*. *Nucleic Acids Res.* 43, 6620–6630. doi: 10.1093/nar/gkv466

Nelis, H. J., and Leenheer, A. P. D. (1991). Microbial sources of carotenoid pigments used in foods and feeds. *J. Appl. Bacteriol.* 70, 181–191. doi: 10.1111/j.1365-2672.1991.tb02922.x

Nichols, L. (n.d.). *Wild Sourdough*. Available online at: http://robdunnlab.com/projects/wildsourdough/ (accessed June 15, 2020).

Oelkers, P. M. (2017). Semester-long inquiry-based molecular biology laboratory: transcriptional regulation in yeast. *Biochem. Mol. Biol. Educ.* 45, 145–151. doi: 10.1002/bmb.21023

Pedwell, R. K., Fraser, J. A., Wang, J. T. H., Clegg, J. K., Chartres, J. D., and Rowland, S. L. (2018). The beer and biofuels laboratory: a report on implementing and supporting a large, interdisciplinary, yeast-focused course-based undergraduate research experience. *Biochem. Mol. Biol. Educ.* 46, 213–222. doi: 10.1002/bmb.21111

PLoS (2019). *Anatomy of an Impossible Burger*. Available online at: https://dnascience.plos.org/2019/05/16/anatomy-of-an-impossible-burger/ (accessed on 27 June 2020).

Saccharomyces Genome Database [SGD] (n.d a) Available at https://www.yeastgenome.org/ (accessed June 15, 2020).

Saccharomyces Genome Database [SGD] (n.d b). *Blog | SGD*. Available online at: https://www.yeastgenome.org/blog/tag/opiate-biosynthesis (accessed June 27, 2020).

Sehgal, N., Sylves, M. E., Sahoo, A., Chow, J., Walker, S. E., and Cullen, P. J. (2018). CRISPR gene editing in yeast: an experimental protocol for an upper-division undergraduate laboratory course. *Biochem Mol. Biol. Educ.* 46, 592–601. doi: 10.1002/bmb.21175

Sitaraman, R. (2011). The treasure of the humble: lessons from Baker's yeast. *Biochem. Mol. Biol. Educ.* 39, 261–266. doi: 10.1002/bmb.20503

Tenenbaum, L. S., Anderson, M. K., Jett, M., and Yourick, D. L. (2014). An innovative near-peer mentoring model for undergraduate and secondary students: STEM focus. *Innov. High. Educ.* 39, 375–385. doi: 10.1007/s10755-014-9286-3

Trujillo, G., Aguinaldo, P. G., Anderson, C., Bustamante, J., Gelsinger, D. R., Pastor, M. J., et al. (2015). Near-peer STEM mentoring offers unexpected benefits for mentors from traditionally underrepresented backgrounds. *Perspect. Undergrad. Res. Mentor. PURM.* 4.

Verwaal, R., Wang, J., Meijnen, J.-P., Visser, H., Sandmann, G., van den Berg, J. A., et al. (2007). High-Level production of beta-carotene in *Saccharomyces cerevisiae* by successive transformation with carotenogenic genes from *Xanthophyllomyces dendrorhous*. *Appl. Environ. Microbiol.* 73, 4342–4350. doi: 10.1128/aem.02759-06

Winzeler, E. A., Shoemaker, D. D., Astromoff, A., Liang, H., Anderson, K., Andre, B., et al. (1999). Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285, 901–906. doi: 10.1126/science.285.5429.901

Wired (n.d.). *Inside the Strange Science of the Fake Meat That 'Bleeds'*. San Francisco, CA: Wired.

Wolyniak, M. J. (2013). Improved student linkage of mendelian and molecular genetic concepts through a yeast-based laboratory module. *Biochem. Mol. Biol. Educ.* 41, 163–172. doi: 10.1002/bmb.20679

Wright, R., Conover, A., and Schnell, R. (2014). The Magic of Doctopus: A quick and easy way to deliver pre-named, pre-shared Google documents directly to your students' Google drive. *Coursesource* 1:14. doi: 10.24918/cs.2014.14

Xie, D., Jackson, E. N., and Zhu, Q. (2015). Sustainable source of omega-3 eicosapentaenoic acid from metabolically engineered *Yarrowia lipolytica*: from fundamental research to commercial production. *Appl. Microbiol. Biotechnol.* 99, 1599–1610. doi: 10.1007/s00253-014-6318-y

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Evaluation of the Impact of the Tiny Earth Project on the Knowledge About Antibiotics of Pre-university Students in the Province of Valencia on Three Different School Years (2017–2020)

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According to the World Health Organization (WHO), antibacterial resistance is a serious problem worldwide. In Spain, knowledge about the use of antibiotics is scarce, being the third country with the highest consumption of antibiotics in the world and the first in Europe. This problem is due, partly, to the abusive use of these drugs in human medicine, livestock, and agriculture. The objective of this study was to evaluate the impact that the Tiny Earth project has had on the antibiotic knowledge in pre-university students. To do this, a survey was conducted before and after the Tiny Earth project in three different school years (2017–2020) to 322 pre-university students belonging to seven schools in the province of Valencia. The survey consisted of 12 multiple-choice questions with a single valid answer. We observed 67.6% success at the beginning and 81.2% at the end. These data indicate that they correctly answered an average of 1.64 more questions after completing the project. In view of the results, we can affirm that the Tiny Earth project has contributed to an improvement in scientific knowledge and awareness of the correct use of antibiotics and the emergence of resistances by pre-university students, which could also be transmitted to their social environment, thus improving awareness global on these issues.

Keywords: Tiny Earth, resistances, antibiotics, rational use, global awareness, knowledge surveys, citizen science

INTRODUCTION

Antibacterial resistance has become a serious health problem worldwide (Prigitano et al., 2018) and is now spreading faster in comparison to the development of new molecules. It should be noted that only 8 of the 33 new antibiotics in development belong to new families, a small number considering the large number of bacterial resistances we are facing (Lewis, 2017). In fact, according to WHO, bacterial resistances will be the leading cause of death in 2050 and the biggest challenge in the field of Biomedicine in the 21st century (World Health Organization, 2016).

In Spain, knowledge about the use of antibiotics is scarce, being the third country with the highest consumption of antibiotics in the world and the first one in Europe (Klein et al., 2018). This problem is due, in part, to the misuse of these drugs in human medicine, livestock, and agriculture (Phillips et al., 2004). This problem is even more serious if we bear in mind that three quarters of the antimicrobial agents used in livestock overlap with antimicrobials used in humans (O'Neill, 2015). This has led to the so-called "antibiotic crisis," result of the shared irresponsibility of health professionals, politicians, and consumers of antibiotics themselves, though in very different degrees of responsibility (Cisneros Herreros and Peñalva Moreno, 2018).

In this context, it seems important to promote educational initiatives on the proper use and prescription of antimicrobial drugs (Scaioli et al., 2015). Following this line of action, Tiny Earth (2018) appears as an innovative project of citizen, participatory, educational, and social science based on a crowdsourcing strategy for the exploration of microbial biodiversity in soils in search of new antibiotic-producing microorganisms (Valderrama et al., 2018). Motivation is reinforced by the fact that there already exist compounds described as antibacterials produced by microorganisms isolated from soil, namely malacidins and teixobactin (Hover et al., 2018). In this way, it motivates pre-university students toward choosing a scientific degree while addressing a global health threat such as the resistance of bacteria to antibiotics (Handelsman et al., 2016; Pernaute and Jiménez, 2017).

A crowdsourcing strategy involves outsourcing tasks, rather than being performed by institution staff, overseeing a large group of volunteers or community through an open call. The idea of crowdsourcing is relatively recent, it shares the perspective defended by some academic sectors of free and open access to scientific knowledge (Open Access) and benefits from advances in communication technologies (internet and social networks). It has been postulated for use in the field of Public Health and other aspects related to Biomedicine (Brabham et al., 2014; Bentzien et al., 2015) and, applied to the educational environment, can provide a collaborative and practical dimension that is highly motivating for students (Caruso et al., 2016).

At the same time that it instructs and motivates, Tiny Earth alerts, reports and raises awareness to society and future generations. It invites pre-university students to participate in a real international project that addresses a very relevant health issue such as the lack of effective antibiotics to fight the infections caused by multi-resistant bacteria, which are already immune to virtually our entire therapeutic arsenal (Davies and Davies, 2010). Throughout the lab lessons, the Tiny Earth program focuses on the idea of discovery, in which students from around the world carry out creative fieldwork and laboratory research on soil samples in search of new antibiotic-producing microorganisms (Davis et al., 2017).

The implantation for the first time at the CEU Cardenal Herrera University (CEU UCH), sponsored by the Spanish Microbiology Society within the D+D group, of the successful educational and informative project of American origin Tiny Earth raises an important and original novelty compared to the United States project. It involves integrating two educational levels, pre-university and university, by implementing an ambitious strategy of service learning, which implies that the teaching activities and strategies used in the training of university students must have a direct impact on the community and society, integrating concepts of active learning, practical teaching, group work, and social volunteering. Indeed, the learning aspect of this project is not limited to the knowledge that pre-university students can acquire, since university students also acquire a series of new skills related to their knowledge transmission skills, teamwork, lab work, and expertise on the subject of antibacterial resistance.

To launch the Tiny Earth project, we recruited undergraduate students who had already taken courses in the field of Microbiology and students in the field of Communication Degrees in various degrees of the CEU UCH, to highlight the importance of divulgation and scientific communication and raise awareness about this health issue. This project was carried out in five private and state-funded schools in the province of Valencia during the first year (2017–2018). The project was expanded the second year (2018–2019) to include two more schools. In its third year (2019–2020), the schools remained the same.

The aim of this study is to evaluate the impact that the Tiny Earth project has had on the knowledge about antibiotics of the participating pre-university students. The results of our survey, which involved people of a very narrow age range, cannot be extended to the entire Spanish population. Nevertheless, they provide valid elements to promote initiatives aimed at a more conscious use of antibiotics.

MATERIALS AND METHODS

The Tiny Earth project consists of five practical sessions, challenging young students to discover novel bioactive-producing microorganisms from soil samples as well as raising awareness about antibiotic resistance and their appropriate use. Throughout 3 academic years, 19 Tiny Earth teams, each consisting of three to five undergraduate students or Tiny Earth Teaching Assistants (Tiny Earth TAs) led by one University teacher or Tiny Earth Partner Instructor (Tiny Earth PI).

To evaluate the pedagogical impact of the project, 322 pre-university students filled in a survey at the beginning (practical 1) and at the end (practical 5) of their participation in the Tiny Earth project, which consisted of 12 multiple-choice questions with one correct answer. We wrote this survey in Spanish and English (**Figure 1**) and we handed it out in one language or the other, depending on the school. The aim of this survey was to assess the level of knowledge about the appropriate use of antibiotics and the level of perception regarding the problem of antibiotic resistances. This survey was prepared based on questions asked by Tiny Earth TAs and Tiny Earth PIs who participated in the project.

Unlike other studies in which respondents were required to provide certain personal data for further demographic analysis (World Health Organization, 2015), in our case, we decided

ANTIBIOTIC KNOWLEDGE SURVEY
Tiny Earth project
Year 2019-20
This survey allows us to know what is your knowledge on antibiotics and their use.
The data you provide is anonymous and confidential.
Ring in (A) the right answer. THERE IS ONLY ONE RIGHT ANSWER.

1. What is the cause of the common cold?
A. Virus
B. Bacteria
C. Getting cold
D. Having your hair wet for a long time
2. Is a prescription necessary to buy antibiotics?
A. Yes, it is always necessary.
B. No, only sometimes (when I have something serious or I do not know what I have).
C. No, a prescription is never necessary.
3. Which of the following drugs is an antibiotic?
A. Aspirin
B. Amoxicillin
C. Paracetamol
D. Ibuprofen
4. Are antibiotics useful for any type of infection?
A. Yes, they can be used to fight any type of microorganism.
B. No, only for infections caused by bacteria, fungi and some parasites.
C. No, only for infections caused by viruses such as the flu.
D. Yes, but it depends on the age of the person infected and if they already have a disease.
5. Can a bacterial infection be treated with any antibiotic?
A. Yes.
B. No.
C. Depends on the age.
D. Depends on the stage at which it was diagnosed.
6. Which of these indications should be kept in mind when taking antibiotics?
A. Medical instructions (schedule and dose) should be followed rigorously.
B. Treatment should be suspended as soon as symptoms disappear.
C. It is important to keep the prescription in order to use it in the future, if the same symptoms appear.
D. All of the above.
7. If we do not take antibiotics correctly:
A. We will get better.
B. We will have problems with the antibiotic resistance that bacteria will probably develop.
C. Animals will become resistant to antibiotics and will never be infected by bacteria.
D. We will use other drugs that "kill bacteria" such as paracetamol, ibuprofen and omeprazole.
8. What do you understand by antibiotic resistance?
A. Antibiotic resistance occurs when microorganisms (bacteria, virus, fungi or parasites) undergo changes, which make the drugs used to treat infections ineffective (WHO Definition).
B. Any mechanism produced by the human body that degrades these drugs, rendering them ineffective and, therefore, lacking therapeutic effect.
C. Processes carried out by bacteria and humans jointly that cause the antibiotic to lose its efficacy for the infection we want to treat.
D. None of the above.
9. Antibiotic-resistant bacteria can be transmitted to humans:
A. Through contact with someone who has an infection caused by antibiotic-resistant bacteria.
B. Through contact with something touched by a person who has an infection caused by antibiotic resistant bacteria (for instance, in hospitals with deficient hygiene, by the hands of health workers or by the instruments they use).
C. Through contact with animals, food or water carrying antibiotic resistant bacteria.
D. All of the above.
10. Which of the following is essential in combating antibiotic resistance?
A. Vaccination
B. Taking antibiotics only under medical prescription, at the correct dose and during the necessary time.
C. Investing in the research of new antibiotics.
D. All of the above.
11. Choose the incorrect statement:
A. I can always recommend someone I know a treatment that I used to treat a bactreial infection. Furthermore, if I had leftover medication, it would be correct to share it with them in order to reduce health expenses.
B. I should never recommend/lend an infection treatment to anyone. In fact, if I had leftovers, I should take them to the nearest pharmacy so they can dispose of them in the correct way.
C. I should never take antibiotics for a viral infection such as the flu or a cold
D. Even if symptoms have disappeared, I will never stop taking antibiotics halfway through the treatment, since that would favor the appearance of antibiotic resistances
12. According to the World Health Organization (WHO), in 2050 it is estimated that the first cause of death will be:
A. Cancer.
B. Infections caused by antibiotic-resistant bacteria.
C. Cardiovascular diseases such as stroke or ictus.
D. Neurodegenerative diseases such as Alzheimer's disease.

FIGURE 1 | Tiny Earth project's survey (in English) consisting of 12 test questions with a single valid answer.

to carry out the surveys anonymously in order not to exert added pressure to students and preserve the relaxed albeit rigorous character of the activities carried out.

Statistical parameters (such as mean, standard deviation, and variances) were calculated using the Microsoft Excel 2016 program. The Fisher-Snedecor test (*F*-test) was used to analyze the equality of the variances and the Student's *t*-test was used to determine statistical significances, both tests performed at 95% confidence interval (CI).

RESULTS

After analyzing the survey results, we observed 67.6% (8.11/12) of success at the beginning and 81.2% (9.74/12) at the end of the project. These data indicate that students correctly answered an average of 1.64 more questions after completing the practical sessions of the project (**Figure 2**). The statistical data reveal that there are significative differences at 95% CI when comparing the overall results at the beginning and at the end of the project (**Table 1**).

When we analyze the results before and after the project, we highlight the following data: to question 1, "What is the cause of the common cold?," 55.0% was correctly answered at the beginning and 64.0% at the end of the practical sessions (**Figure 3**). To question 2, "Is a prescription necessary to buy antibiotics?," 62.1% was correctly answered at the beginning and 90.6% at the end of the practical sessions (**Figure 4**). To question 4, "Are antibiotics useful for any type of infection?," 70.2% was correctly answered at the beginning and 83.2% at

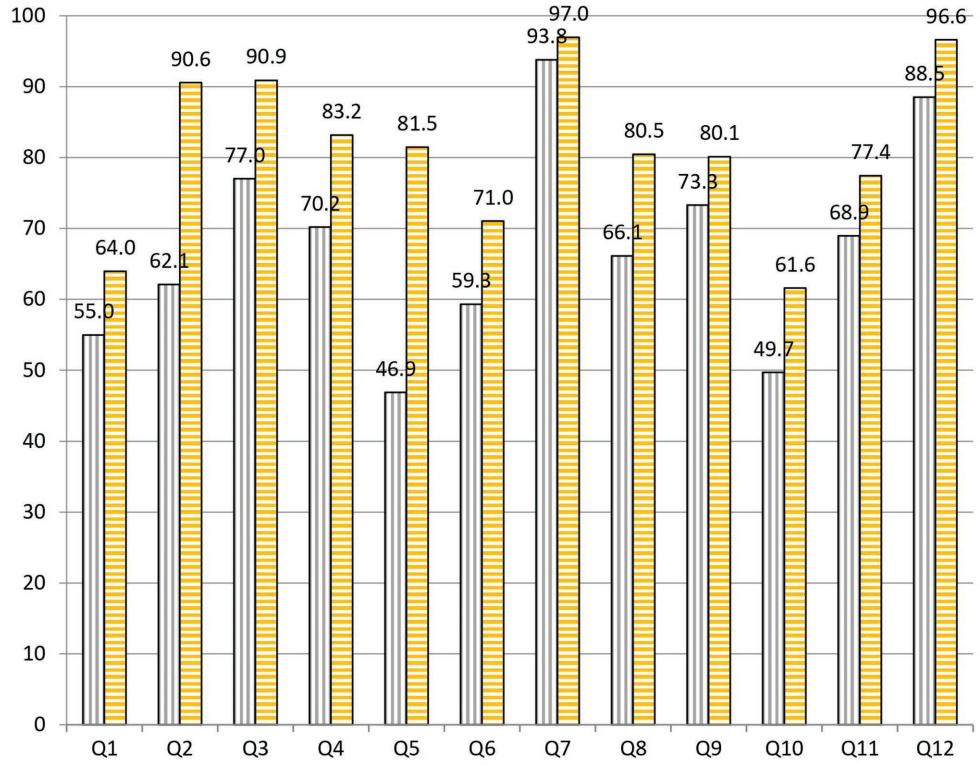

FIGURE 2 | Percentages of correct answers to the 12 questions of the test carried out by pre-university students at the start (purple bars with vertical stripes) and at the end (yellow bars with horizontal stripes) of the Tiny Earth project during the courses 2017–2020.

the end of the practical sessions (**Figure 5**). To question 5, "Can a bacterial infection be treated with any antibiotic?," 46.9% was correctly answered at the beginning and 81.5% at the end of the practical sessions (**Figure 6**). To question 6, "Which of these indications should be kept in mind when taking antibiotics?," 59.3% was correctly answered at the beginning and 71.0% at the end of the practical sessions (**Figure 7**). To question 8, "What do you understand by antibiotic resistance?," 66.1% was correctly answered at the beginning and 80.5% at the end of the practical sessions (**Figure 8**). To question 10, "Which of the following is essential in combating antibiotic resistance?," 49.7% was correctly answered at the beginning and 61.6% at the end of the practical sessions (**Figure 2**). The differences in the results before and after the project have proven to be statistically significant at 95% CI for questions 2 and 8 (**Table 1**).

DISCUSSION

From the results of the survey, in which there has been an increase close to 14% when comparing the number of questions answered correctly at beginning and at the end of the sessions, we can affirm that there has been a significant overall improvement in scientific knowledge from pre-university students participating in this project.

Nevertheless, we do believe that the results obtained in some of the questions asked deserve special attention, either because the difference in the correct answers to a specific question has been negligible, or because this difference has been especially noteworthy.

In question 1, "What is the cause of common cold?," a slight drop (1.1%) was detected between the answers at the beginning (50.0%) and at the end (48.9%) of the project during the course 2017–2018 (**Figure 3**). A possible explanation for these data would be that, having carried out sessions dedicated mainly to bacteria, it might had influenced the perception that diseases are always caused by bacteria. We felt that the results on question 1 were too low on the first course, so in later editions, we tried to deepen our explanation regarding other diseases caused by other microorganisms, which gave place to a significant rise in the percentage of correct answers (10.0% in 2018–2019 and 17.1% in 2019–2020). We could say that the explanation was more efficient or effective in the following courses since, without changing it, it was more emphasized and better understood.

Question 2, "Is a prescription necessary to buy antibiotics?," underwent a very considerable change with a 28.5% overall increase in correct answers given before and after the project (**Figure 4**). These data are probably due to the emphasis shown by Tiny Earth TAs and Tiny Earth PIs about the risks, both for the patient and for society, associated with the sale of

TABLE 1 | Statistical parameters at 95% confidence interval of the comparison between results obtained at the start and at the end of the project.

	Standard deviation	Variance	*F*-test	Variance equality	Weighted variance	*t*-test	Significative differences
Q1 start	5.048	16.988	5.855	Equal	87.345	1.131	No
Q1 end	12.215	99.472					
Q2 start	11.058	81.518	5.861	Equal	71.570	4.200	Yes
Q2 end	4.568	13.909					
Q3 start	10.005	66.735	15.951	Equal	53.189	2.378	No
Q3 end	2.505	4.184					
Q4 start	11.192	83.504	8.390	Equal	70.093	1.997	No
Q4 end	3.864	9.953					
Q5 start	37.219	923.491	144.997	Unequal	697.395	2.133	No
Q5 end	3.091	6.369					
Q6 start	5.698	21.645	18.052	Equal	309.290	0.783	No
Q6 end	24.210	390.741					
Q7 start	2.211	3.259	5.388	Equal	2.898	2.481	No
Q7 end	0.952	0.605					
Q8 start	4.018	10.765	2.120	Equal	25.188	3.512	Yes
Q8 end	5.850	22.818					
Q9 start	7.473	37.235	1.726	Equal	44.104	1.190	No
Q9 end	5.688	21.569					
Q10 start	13.975	130.197	2.380	Equal	138.681	1.265	No
Q10 end	9.059	54.711					
Q11 start	13.293	117.802	3.371	Equal	114.561	1.020	No
Q11 end	7.240	34.946					
Q12 start	5.389	19.358	15.928	Equal	15.430	2.327	No
Q12 end	1.350	1.215					
Overall start	3.818	9.717	9.165	Equal	8.083	5.998	Yes
Overall end	1.261	1.060					

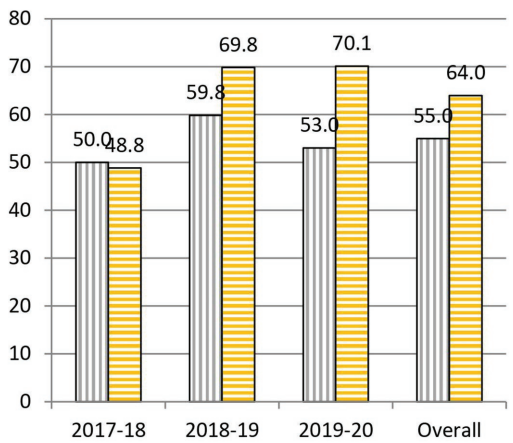

FIGURE 3 | Percentages of correct answers to Question 1 "What is the cause of the common cold?" at the start (purple bars with vertical stripes) and at the end (yellow bars with horizontal stripes) of the Tiny Earth project during the courses 2017–2020.

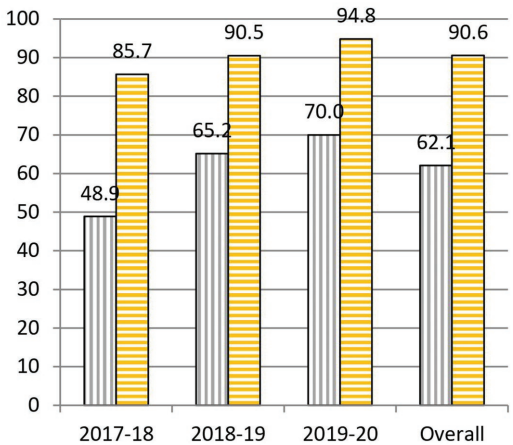

FIGURE 4 | Percentages of correct answers to Question 2 "Is a prescription necessary to buy antibiotics?" at the start (purple bars with vertical stripes) and at the end (yellow bars with horizontal stripes) of the Tiny Earth project during the courses 2017–2020.

antibiotics without a prescription (Guinovart et al., 2018). We would like to highlight that, year after year, the percentage of correct answers at the end has not ceased to increase (85.7% in 2017–2018, 90.5% in 2018–2019, and 94.8% in 2019–2020). Thus, we can conclude that the tendency of the gap between the percentage of correct answers before and after the project to shrink has more to do with the fact that students had a better knowledge on this question to begin with (48.9% of correct answers at the beginning of course 2017–2018, 65.2% in 2018–2019, and 70.0% in 2019–2020).

Another question that underwent a remarkable change, with a 13.0% increase in correct answers before and after the project was question 4, "Are antibiotics useful for any type of infection?" (**Figure 5**). Behind this improvement could be the continuous reminder by Tiny Earth TAs and Tiny Earth PIs throughout

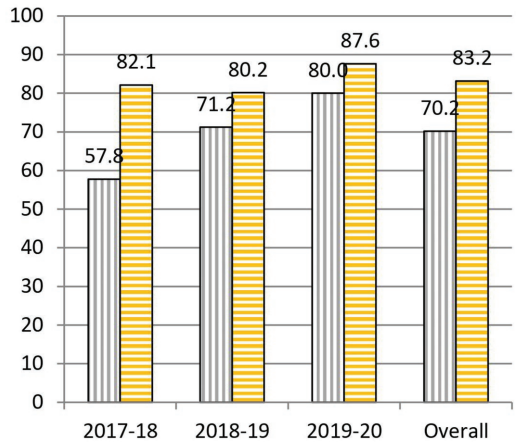

FIGURE 5 | Percentages of correct answers to Question 4 "Are antibiotics useful for any type of infection?" at the start (purple bars with vertical stripes) and at the end (yellow bars with horizontal stripes) of the Tiny Earth project during the courses 2017–2020.

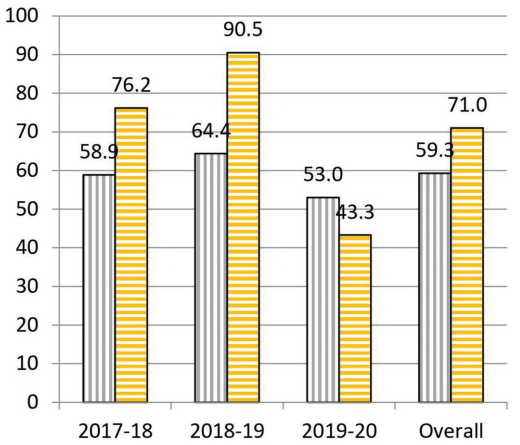

FIGURE 7 | Percentages of correct answers to Question 6 "Which of these indications should be kept in mind when taking antibiotics?" at the start (purple bars with vertical stripes) and at the end (yellow bars with horizontal stripes) of the Tiny Earth project during the courses 2017–2020.

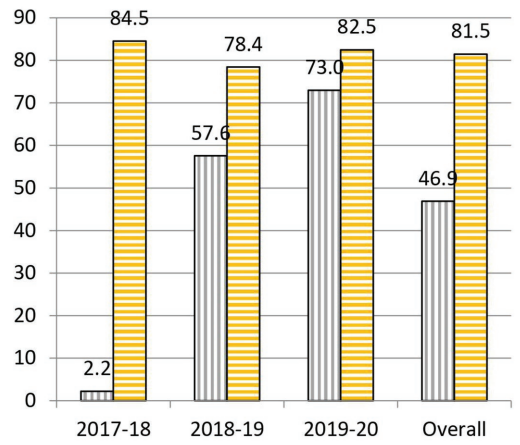

FIGURE 6 | Percentages of correct answers to Question 5 "Can a bacterial infection be treated with any antibiotic?" at the start (purple bars with vertical stripes) and at the end (yellow bars with horizontal stripes) of the Tiny Earth project during the courses 2017–2020.

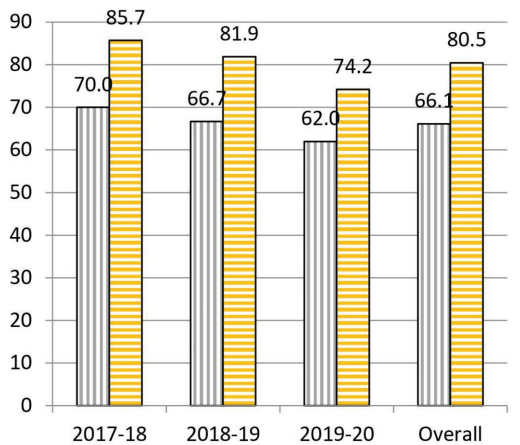

FIGURE 8 | Percentages of correct answers to Question 8 "What do you understand by antibiotic resistance?" at the start (purple bars with vertical stripes) and at the end (yellow bars with horizontal stripes) of the Tiny Earth project during the courses 2017–2020.

all practical sessions that antibiotics are indeed not useful for all infections. Similarly to question 2, even though the percentage of correct answers at the end of the project was quite high (82.1% in 2017–2018, 80.2% in 2018–2019, and 87.6% in 2019–2020), the general knowledge about this question before the project grew year after year (57.8% in 2017–2018, 71.2% in 2018–2019, and 80.0% in 2019–2020), reason why yearly gaps have decreased.

In question 5, "Can a bacterial infection be treated with any antibiotic?," there has been a striking increase (34.6%) in the percentage of correct answers before and after the project (**Figure 6**). This could be due to the emphasis shown during the project, in which we explained that not all bacteria are alike, that they can cause various and very different infectious diseases, in addition to the own experience that pre-university students have had when viewing the different degrees of antibiosis against the same bacteria in the lab. A similar pattern to question 4 has been obtained. The percentage of correct answers at the end of the project were quite high (84.5% in 2017–2018, 78.4% in 2018–2019, and 82.5% in 2019–2020), the general knowledge about this question before the project grew year after year (2.2% in 2017–2018, 57.6% in 2018–2019, and 73.0% in 2019–2020), reason why yearly gaps have also decreased.

In question 6, "Which of these indications should be kept in mind when taking antibiotics?," there has been an overall increase of 11.7% correctly answered questions between the beginning and the end of the practical sessions (**Figure 7**). However, if we focus on the yearly data, very good results were obtained in the first two courses (17.3% increase in 2017–2018 and 26.1% in 2018–2019), but there was a significant drop (9.7%) of correct answers between the beginning and the end of this course's project. This result serves as a lesson to not to forget the importance of the rational use of antibiotics. Therefore, we should put a stronger emphasis on this matter in the coming years.

In question 8, "What do you understand by antibiotic resistance?," there has been an overall increase of 14.3% in correct answers from the beginning to the end of the practical sessions (**Figure 8**). One of the reasons for these good results might be, besides the theoretical explanations on the concept of resistance given throughout the project, the fact that they have been able to recognize resistances visually by observation of inhibition halos.

In question 10, "Which of the following is essential in combating antibiotic resistance?," there has been an overall increase of 11.9% in correct answers from the beginning to the end of the practical sessions (**Figure 2**). Even though there have been questions with less improvement between the beginning and the end, it is the question with the lowest success rate at the end of the project (61.6%). This might be due to the fact that it is a question in which all the answers are correct and, therefore, they must have learned all the concepts stated in the question.

We must say that our first goal was not to obtain uniform results from the tests. However, after analyzing the results on our first year, we tried to do some improvements in our presentations based on that information and, thus, results became more uniform for the last 2 years. However, we sincerely hope that teachers who may want to start a similar project could benefit from our findings.

We should also note that in schools where the number of students has been lower, both the results and collaboration have been more positive because the sessions were held more calmly and with a more personalized attention. In schools where there have been more students, although the results have also been satisfactory, attention could not have been so individualized. These results are consistent with various studies showing that while a significant proportion of the general public still believe that antibiotics are an effective treatment for cold symptoms, they also report increased awareness of antibiotic resistance (European Commission, 2013; Carter et al., 2016).

Chronic infectious diseases such as HIV and tuberculosis and emerging infections with the potential for rapid expansion such as the 2014 Ebola epidemic and the 2020 COVID-19 pandemic remain a substantial global health threat to mankind (Shahmanesh et al., 2020). Infectious diseases have profound effects beyond health, especially on local and global economies, which intensifies existing socioeconomic fragilities (United Nations Development Programme and International Federation of Red Cross and Red Crescent Societies, 2017). However, the prevalence of antimicrobial resistance grows by the year and we will soon be speaking about millions of deaths from antibiotic-resistant infections (Collignon et al., 2018).

We think that there is an urgent need for educational and awareness programs integrating methods to optimize the prevention of, and response to, infectious diseases. This might mean training science teachers and broadcasters to improve their dissemination on human health (Samet and Woodward, 2019).

CONCLUSION

The global experience of the Tiny Earth project has been very positive. Participation in the Tiny Earth project has positively contributed to the development of scientific interest, the awareness on the proper use of antibiotics, and the emergence of resistances in pre-university studies, as well as improved their skills in the laboratory.

Likewise, in view of the results obtained with the survey, we can affirm that the students participating in this project have improved their scientific knowledge. Besides, these newly learned skills may also be transmitted to their social environment, thus improving the overall awareness of this issue. We can also conclude that the Tiny Earth project has awakened and expanded the scientific training and interest in students.

We would also like to highlight the need for new approaches in order to reach the general public in the field of infectious diseases.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, and further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical approval was not provided for this study on human participants because no personal data was collected. We asked for informed consent given that the students that participated in the project where under 18 years old. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

M-TP-G was responsible for the project design, conception and management and the integrity of the work, and overall supervision. JB-B, BS-G, CG-R, and M-TP-G performed the practicals. EM-C was responsible for the broadcasting of the project. JB-B wrote the first draft of the manuscript. BS-G, CG-R, and EM-C wrote sections of the manuscript.

All authors contributed to interpretation of the data, manuscript revision, read, and approved the manuscript.

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REFERENCES

Bentzien, J., Bharadwaj, R., and Thompson, D. C. (2015). Crowdsourcing in pharma: a strategic framework. *Drug Discov. Today* 20, 874–883. doi: 10.1016/j.drudis.2015.01.011

Brabham, D. C., Ribisl, K. M., Kirchner, T. R., and Bernhardt, J. M. (2014). Crowdsourcing applications for public health. *Am. J. Prev. Med.* 46, 179–187. doi: 10.1016/j.amepre.2013.10.016

Carter, R. R., Sun, J., and Jump, R. L. (2016). A survey and analysis of the American Public's perceptions and knowledge about antibiotic resistance. *Open Forum Infect. Dis.* 3:ofw112. doi: 10.1093/ofid/ofw112

Caruso, J. P., Israel, N., Rowland, K., Matthew, J., Lovelace, M. J., and Saunders, M. J. (2016). Citizen science: the Small World Initiative improved lecture grades and California critical thinking skills test scores of nonscience major students at Florida Atlantic University. *J. Microbiol. Biol. Educ.* 17, 156–162. doi: 10.1128/jmbe.v17i1.1011

Cisneros Herreros, J. M., and Peñalva Moreno, G. (2018). La crisis de los antibióticos: profesionales sanitarios, ciudadanos y políticos, todos somos responsables. *Enferm. Infecc. Microbiol. Clin.* 36, 259–261. doi: 10.1016/j.eimc.2018.02.002

Collignon, P., Beggs, J. J., Walsh, T. R., Gandra, S., and Laxminarayan, R. (2018). Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. *Lancet Planet. Health* 2, e398–e405. doi: 10.1016/S2542-5196(18)30186-4

Davies, J., and Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* 74, 417–433. doi: 10.1128/MMBR.00016-10

Davis, E., Sloan, T., Aurelius, K., Barbour, A., Bodey, E., Clark, B., et al. (2017). Antibiotic discovery throughout the Small World Initiative: a molecular strategy to identify biosynthetic gene clusters involved in antagonistic activity. *MicrobiologyOpen* 6:e00435. doi: 10.1002/mbo3.435

European Commission (2013). Special Eurobarometer 407. Antimicrobial resistance. Report. Available at: http://ec.europa.eu/health/antimicrobial_resistance/docs/ebs_407_en.pdf (Accessed April 15, 2020).

Guinovart, M. C., Figueras, A., and Llor, H. (2018). Selling antimicrobials without prescription—far beyond an administrative problem. *Enferm. Infecc. Microbiol. Clin.* 36, 290–292. doi: 10.1016/j.eimc.2016.10.006

Handelsman, J., Tsang, T., and Hernandez, S. (2016). *Small World Initiative: A research guide to microbial and chemical diversity*. New Haven: Small World Initiative Press.

Hover, B. M., Kim, S. H., Katz, M., Charlop-Powers, Z., Owen, J. G., Ternei, M. A., et al. (2018). Culture-independent discovery of the malacidins as calcium-dependent antibiotics with activity against multidrug-resistant Gram-positive pathogens. *Nat. Microbiol.* 3, 415–422. doi: 10.1038/s41564-018-0110-1

Klein, E. Y., Van Boeckel, T. P., Martinez, E. M., Pant, S., Gandra, S., Levin, S. A., et al. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proc. Natl. Acad. Sci. U. S. A.* 115, E3463–E3470. doi: 10.1073/pnas.1717295115

Lewis, K. (2017). New approaches to antimicrobial discovery. *Biochem. Pharmacol.* 134, 87–98. doi: 10.1016/j.bcp.2016.11.002

O'Neill, J. (2015). Antimicrobials in agriculture and the environment: reducing unnecessary use and waste. The review on antimicrobial resistance. Available at: https://amr-review.org/sites/default/files/Antimicrobials%20in%20agriculture%20and%20the%20environment%20-%20Reducing%20unnecessary%20use%20and%20waste.pdf (Accessed April 15, 2020).

Pernaute, L., and Jiménez, V. (2017). Small World Initiative: educando para combatir la resistencia a los antibióticos. PharmaTech 32, 74–80. Available at: https://www.pharmatech.es/kiosco/revista32/visor (Accessed April 22, 2020).

Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., et al. (2004). Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *J. Antimicrob. Chemother.* 53, 28–52. doi: 10.1093/jac/dkg483

Prigitano, A., Romanò, L., Auxilia, F., Castaldi, S., and Tortoano, A. M. (2018). Antibiotic resistance: Italian awareness survey 2016. *J. Infect. Public Health* 11, 30–34. doi: 10.1016/j.jiph.2017.02.010

Samet, J. M., and Woodward, A. (2019). On being an epidemiologist. *Am. J. Epidemiol.* 188, 818–824. doi: 10.1093/aje/kwy279

Scaioli, G., Gualano, M. R., Gili, R., Masucci, S., Bert, F., and Siliquini, R. (2015). Antibiotic use: a cross-sectional survey assessing the knowledge, attitudes and practices amongst students of a school of medicine in Italy. *PLoS One* 10:e0122476. doi: 10.1371/journal.pone.0122476

Shahmanesh, M., Harling, G., Coltart, C. E. M., Bailey, H., King, C., Gibbs, J., et al. (2020). From the micro to the macro to improve health: microorganism ecology and society in teaching infectious disease epidemiology. *Lancet Infect. Dis.* 20, e142–e147. doi: 10.1016/S1473-3099(20)30136-5

Tiny Earth (2018). Available at: https://tinyearth.wisc.edu (Accessed June 22, 2020).

United Nations Development Programme and International Federation of Red Cross and Red Crescent Societies (2017). A socio-economic impact assessment of the Zika virus in Latin America and the Caribbbean: with a focus on Brazil, Colombia and Suriname. Available at: http://www.undp.org/content/dam/undp/library/HIV-AIDS/UNDP-Zika-04-03-2017-English-WEB.pdf (Accessed June 11, 2020).

Valderrama, M. J., González-Zorn, B., de Pablo, P. C., Díez-Orejas, R., Fernández-Acero, T., Gil-Serna, T., et al. (2018). Educating in antimicrobial resistance awareness: adaptation of the Small World Initiative program to service-learning. *FEMS Microbiol. Lett.* 365:fny161. doi: 10.1093/femsle/fny161

World Health Organization (2015). Antibiotic resistance: multi-country public awareness survey. Available at: http://apps.who.int/iris/bitstream/10665/194460/1/9789241509817eng.pdf?ua=1 (Accessed April 22, 2020).

World Health Organization (2016). Meeting the challenge of antimicrobial resistance. Available at: https://www.who.int/antimicrobial-resistance/interagency-coordination-group/IACG_Meeting_challenge_AMR_communication_to_collective_action_270718.pdf (Accessed April 22, 2020).

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Propelling a Course-Based Undergraduate Research Experience Using an Open-Access Online Undergraduate Research Journal

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The University of British Columbia has developed a course-based undergraduate research experience (CURE) that engages students in authentic molecular microbiology research. This capstone course is uniquely built around an open-access online undergraduate research journal entitled Undergraduate Journal of Experimental Microbiology and Immunology (UJEMI). Students work in teams to derive an original research question, formulate a testable hypothesis, draft a research proposal, carry out experiments in the laboratory, and publish their results in UJEMI. The CURE operates in a feed forward manner whereby student-authored UJEMI publications drive research questions in subsequent terms of the course. Progress toward submission of an original manuscript is scaffolded using a series of communication assignments which facilitate formative development. We present a periodic model of our CURE that guides students through a research cycle. We review two ongoing course-based projects to highlight how UJEMI publications prime new research questions in the course. A journal-driven CURE represents a broadly applicable pedagogical tool that immerses students in the process of doing science.

Keywords: course-based undergraduate research experience, undergraduate research journal, scientific enculturation, pedagogy, curriculum, molecular microbiology, STEM-science technology engineering mathematics

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INTRODUCTION

Becoming a scientist is a complex endeavor that requires multiple levels of development. An essential goal for any successful undergraduate program in STEM is to provide opportunities for students to develop skills in the context of being able to do real-world science (Laursen et al., 2010; American Association for the Advancement of Science, 2011; Feldman et al., 2013). To support students as scientists in training, activities in the curriculum ought to ensure that students acquire technical skills, the ability to read and interpret scientific literature, learn how to design experiments, document observations, analyze and interpret data, and have the opportunity to disseminate research findings (Coil et al., 2010). These fundamental skills form a foundation to support higher order activities including innovation, teamwork, self-authorship, expert thinking, collaboration, and meaningful engagement with the scientific community. Collectively, this developmental process can be described as scientific enculturation (Florence and Yore, 2004; Auchincloss et al., 2014; Linn et al., 2015).

Scientific enculturation requires opportunities for students to "do science" (Linn et al., 2015). Research experiences and mentorship from scientists are needed for students to acquire on the ground training, disciplinary knowledge and understanding of the scientific method (Linn et al., 2015; Estrada et al., 2018). Several types of undergraduate research opportunities have been documented to provide a range of different scientific experiences (Lopatto, 2004, 2010; Seymour et al., 2004; Russell et al., 2007; Sadler et al., 2010; Linn et al., 2015; Robnett et al., 2015). Credit-based undergraduate research opportunities include protocol-driven teaching laboratories, inquiry-based teaching laboratories such as course-based undergraduate research experience (CUREs), and research internships. While protocol-driven teaching labs generally involve activities where the experimental results are known at the outset, at least to the instructor (Weaver et al., 2008), CUREs and research internships tend to address novel research questions where the experimental outcome is usually unknown (Auchincloss et al., 2014; Beck et al., 2014). Research internships, often called directed studies or honors thesis projects, typically have a one-to-one structure where an undergraduate student mentee is paired with a more senior scientist as a mentor (Shapiro et al., 2015). With capable mentors, internships can provide high quality research experiences; however, because mentor:mentee pairings tend to be self-selecting, student diversity, and equitable access can be limited (Bangera and Brownell, 2014). In contrast, CUREs are designed to be scalable and accessible by accommodating a few to several hundred student mentees to one or more faculty mentors (Linn et al., 2015). The course-based nature of CUREs also means that lectures and tutorials can be paired with research activities to provide consistent training in fundamental research skills. CUREs are a rapidly growing pedagogical model for teaching science curricula to promote enculturation and scientific identity among all students in a program, and not an exclusive few (Bangera and Brownell, 2014; Esparza et al., 2020).

Auchincloss et al. (2014) proposed that science-based CUREs can be defined by five main domains in which students: (1) engage in scientific practices, which include technical skills development and the use of the scientific method, (2) experience discovery, as the outcome of an experiment is not known by the students or the instructor at the outset, (3) pursue research questions with broad relevance and meaning beyond the classroom setting, (4) collaborate with their peers, as fellow scientists, and sometimes with practicing scientists in the broader community, and (5) iterate, as experiments are repeated, refined, and cross-examined to generate more robust models and concrete knowledge. Taken together, these domains provide students with an experience that integrates the complex facets of doing authentic research (Brownell et al., 2015). As a result, positive outcomes of CUREs on student development have been documented in several areas of research competency including science identity and confidence, content knowledge, and science literacy (Brownell et al., 2015; Olimpo et al., 2016).

A broad range of science-based CUREs have been developed within disciplines (e.g., biology, chemistry, physics, mathematics, geography) as well as across disciplines (Brownell et al., 2015; Kerr and Yan, 2016; Sarmah et al., 2016; Shanle et al., 2016; Alford et al., 2017; Ballen et al., 2017; Ayella and Beck, 2018; Light et al., 2019; Shelby, 2019; Stoeckman et al., 2019; Wolkow et al., 2019). Bhattacharyya et al. (2020) showcase the wide range of diversity in CURE design (Bhattacharyya et al., 2020). Some CURE courses focus on one main biological model such as expression of p53 tumor suppressor gene in yeast (Brownell et al., 2015), protein interactions with Mer tyrosine kinase (Shelby, 2019), mutagenesis of lactate dehydrogenase (Ayella and Beck, 2018), or the effect of nicotine and caffeine on the development of zebrafish (Sarmah et al., 2016). Some involve students collaborating with an outside institution to conduct their research projects such as the Rosetta Research Experience for Undergraduates where students undertake their CUREs outside of the institution following a 2 weeks programming boot camp (Alford et al., 2017). Others involve consecutive CURE courses taken throughout a student's degree that progressively building a single research topic (e.g., antibiotic resistance) (Light et al., 2019).

Here we review a capstone CURE developed at the University of British Columbia that centers around student-driven microbiology-based projects, and culminates in the generation of original research articles published in an online undergraduate research journal titled the Undergraduate Journal of Experimental Microbiology and Immunology (UJEMI). We describe the structure and function of our CURE model and discuss UJEMI as a tool with the potential to objectively assess student development and observe the process of scientific enculturation. We hope that insights gleaned from our experiences may be helpful to others seeking to design and understand the pedagogical value of CUREs.

AN UNDERGRADUATE RESEARCH JOURNAL-DRIVEN CURE

The University of British Columbia's (UBC) Point Grey campus located in Vancouver, Canada is a large research intensive post-secondary institution which serves over 45,000 undergraduate students and 10,000 graduate students annually (The University of British Columbia, 2020). Since 2001, UBC has been developing a capstone molecular microbiology CURE that serves students in the final year of their 4 year undergraduate program offered by the Department of Microbiology and Immunology. Initially starting out as an optional course enrolling a few students, the course is now required for graduation and has grown to accommodate up to 60 students per semester, totaling approximately 120 students per academic year. Prior to enrolling in the CURE, students are required to complete two lab courses. One is a traditional, protocol-driven lab while the other is a guided inquiry-based lab; together they provide students with fundamental knowledge and skills required to begin working independently in a molecular microbiology laboratory. Resourced with a single instructor and one to two graduate student teaching assistants, the CURE unfolds over 16 weeks (September–December or January–April).

The course is equipped with four learning centers: an interactive classroom lecture (1.5–3 h per week), a team-based meeting (1 h per week), web-based resources including

classroom management tools for communication and an open-access undergraduate research journal[1], and a wet-bench research laboratory outfitted with the majority of the tools necessary to conduct microbiology and molecular biology which is open to students throughout the week. In addition, students are encouraged to interact with researchers working in grant-funded laboratories at UBC, which increases the scope and breadth of the scientific and technological resources available in the course.

The primary instructor of the CURE manages our undergraduate research journal, UJEMI. The structure and function of UJEMI have been previously described (Sun et al., 2020). Briefly, the CURE instructor mentors graduate student editors employed as teaching assistants over the summer months to administer a student-centered peer-review experience, and prepare the manuscripts for online publication (Sun et al., 2020). In addition to papers generated from research conducted in our CURE, UJEMI invites submissions from undergraduate students doing scientific research projects in microbiology and/or immunology at accredited universities around the world. Taken together, UJEMI provides a platform for undergraduate researchers to participate in the authentic process of research dissemination as published authors, and the novel findings published as UJEMI articles drive new research questions in our CURE term after term.

Our CURE is divided into three phases where students engage in planning, experimentation, and dissemination, respectively (**Figure 1**). Writing assignments are used to scaffold the process and provide clear milestones as the course unfolds.

The planning phase (weeks 1–6) begins by directing students to papers published in UJEMI by former students in the course. Students review the UJEMI literature and consider the data and proposed models. Students are encouraged to link their reading to the broader literature. Based on their reading, individual students submit a flowchart as well as a 1-page letter of intent explaining their proposed research question, hypothesis, experimental questions, and potential outcomes. Students also present a brief feasibility analysis. Written feedback on each proposal is provided by the instructor and teaching assistant(s) (week 2). Teaching assistants in the CURE are typically senior graduate students with backgrounds in microbiology and/or immunology who have a demonstrated aptitude for experimental research and teaching.

During the planning phase, students self-assemble into teams of 3 or 4 people and are assigned a weekly meeting time. Each team evaluates each individual proposal and selects a lead project to carry forward for the term. The team's decision considers the potential scientific impact of the project, areas of development that individuals or the team would like to pursue (e.g., experience with a specific technique), feasibility, and the risk to reward ratio. The course learning objectives do not include a prescribed set of techniques that the students must learn; rather the focus is on working through a hypothesis-driven research project using the tools best suited to address the research question.

The team then moves into a series of team meetings in which the lead proposal is developed (weeks 2–6). The instructor and teaching assistants provide guidance as students refine their proposal to ensure that their hypothesis is testable, that their experiments are well designed and technically feasible, that they are sourcing reliable protocols and methods, and that they create strategies to execute their project within the constraints of the course (e.g., time, resources, expertise). It is important to note that the instructor and teaching assistants do not direct project development, but instead facilitate the process. The students are expected to guide their own research direction, which promotes project ownership. The development of a novel research question and self-directed approach to project management are elements of CUREs that have shown to increase student perceptions of ownership over their own projects and the outcomes associated with their projects (Cooper et al., 2019). This planning phase concludes with submission and feedback on an extensive team-based proposal that details the scientific background, hypothesis, experimental aims, protocols, and methods, laboratory safety considerations, a timeline, and pitfalls and workarounds (week 8). The team proposal becomes a road map for the project.

The experimentation phase is carried over weeks 6–12. Student teams are assigned a lab bay and prepare their own reagents including stock solutions and growth media. They also design primers and culture lab-based *Escherichia coli* strains which they can request from the course strain collection, the Coli Genetic Stock Center at Yale University, or from academic researchers around the world who have published strain descriptions. Students plan their own lab work schedules and are encouraged to divide the work amongst their team members so as not to over burden any one individual. The lab is open during the week from approximately 8a.m. to 5p.m. and students come and go during the day. Although we don't monitor the time spent working on the project, student teams are given the same explicit deadlines. We estimate that individual students spend approximately 4–6 h per week working on their project which, if equitably distributed across their team, accounts for about 16–24 h of team-based lab work per week. Instructors and teaching assistants are available for guidance and demonstrations of technical steps. Similar to most research experiences, experiments rarely work on the first attempt and students often repeat steps before achieving a result. Bi-weekly written research reports and team meetings are used for reflection and feedback (weeks 9, 11, and 13). Students are often able to troubleshoot their own experiments after systematic reflection in written form. The experimentation phase concludes with an oral presentation to the class summarizing their research question and findings (week 12). Peer- and instructor-based feedback is gathered to support the dissemination phase.

The final phase involves dissemination of research results in the form of an original research article (weeks 12–16). Building off instructor and peer feedback from their oral presentation, as well as classroom activities in which strategies for drafting an original research manuscript are discussed, the students assemble their data as figures and tables and attempt to construct a coherent story. Instructors and teaching assistants provide guidance especially with deeper data analysis and reaching well-supported conclusions to provide students with enough scaffolding to facilitate the drafting process. Student teams

[1]https://ujemi.microbiology.ubc.ca/

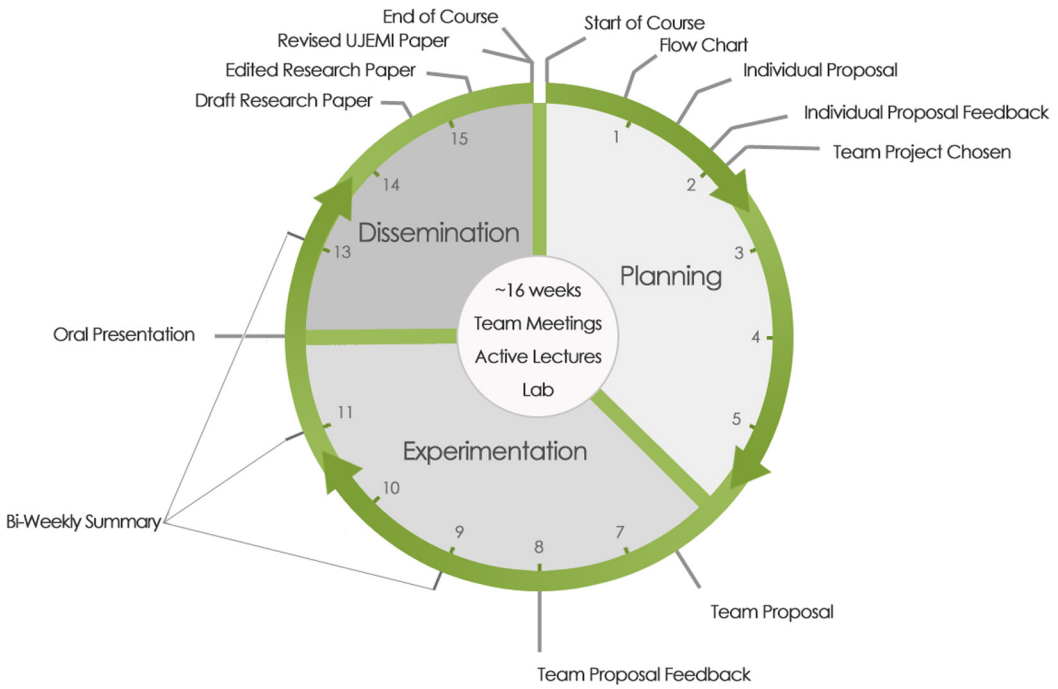

FIGURE 1 | Research cycle over a 16 week academic term. Planning, experimentation, and dissemination phases are denoted. Due dates for communication assignments and feedback scaffolding the CURE are shown on the periphery. Individual proposals are submitted after the first week of classes. The team proposal is submitted at the conclusion of the planning phase in week 6. Teams conduct oral presentations on week 12 at the beginning of the dissemination phase. Draft manuscripts are submitted at the end of week 14 and the final manuscript just before the end of the course around week 15.

submit a draft manuscript, formatted as per the Instructions for Authors guidelines set out by the Journal of Bacteriology. The manuscripts are reviewed by the instructor and teaching assistants (week 15) and returned to the student teams for revision (week 16). Students revise their work (often extensively) prior to final submission and provide a response to reviewers (week 16). A course grade is not assigned until the paper is accepted for publication in UJEMI. Students have the option of advancing their manuscript to a peer review phase if their work communicates a *bona fide* well-controlled finding (either negative or positive data). The peer review process extends beyond the end of the course (Sun et al., 2020). Importantly, papers published in UJEMI serve as fuel for the next iteration of the course, and the research cycle continues.

CONNECTING RESEARCH PROJECTS

Over 4 months, student teams work through a research cycle (**Figure 1**). The publication of a UJEMI article creates a body of knowledge that can be used to derive new research questions. A broad range of projects have been developed by students in the course that span the fields of molecular biology, biochemistry, and microbiology. Projects include research on bacteriophage (Chiu et al., 2017; Dimou et al., 2019), bacteria (Cramb et al., 2015; Backstrom et al., 2017; Hartstein et al., 2017), and yeast (Goldhawke et al., 2016), as well as *Caenorhabditis elegans* as a model host organism (James et al., 2018; Cheng et al., 2019). Students have employed a wide range of microbiology and molecular biology techniques including standard PCR, quantitative PCR, Gibson's cloning, flow cytometry, and Next Generation Sequencing. As of 2019, UJEMI had published 493 original research articles solely authored by undergraduate students. Individual articles investigating common research questions can be clustered into ongoing course-based projects. Two ongoing research projects are summarized in **Supplementary Table S1** and are mapped chronologically in **Figure 2**. We review these two projects as case studies to provide insight into how research evolves over multiple terms of the course.

THE *WZA* STORY: ANTIBIOTIC SUSCEPTIBILITY AND CAPSULE SECRETION GENES

It has been suggested that capsule, a discrete layer of polysaccharide linked to the cell surface of some bacteria including *E. coli*, could create a physical barrier to impede the movement of molecules such as antibiotics into the cell (Slack and Nichols, 1982). Decreased intracellular concentration of the antibiotic may result in tolerance to high extracellular concentration of the antibiotic (i.e., increased resistance). Several mechanisms of capsule mediated resistance have been proposed including the idea that charged-based interactions between

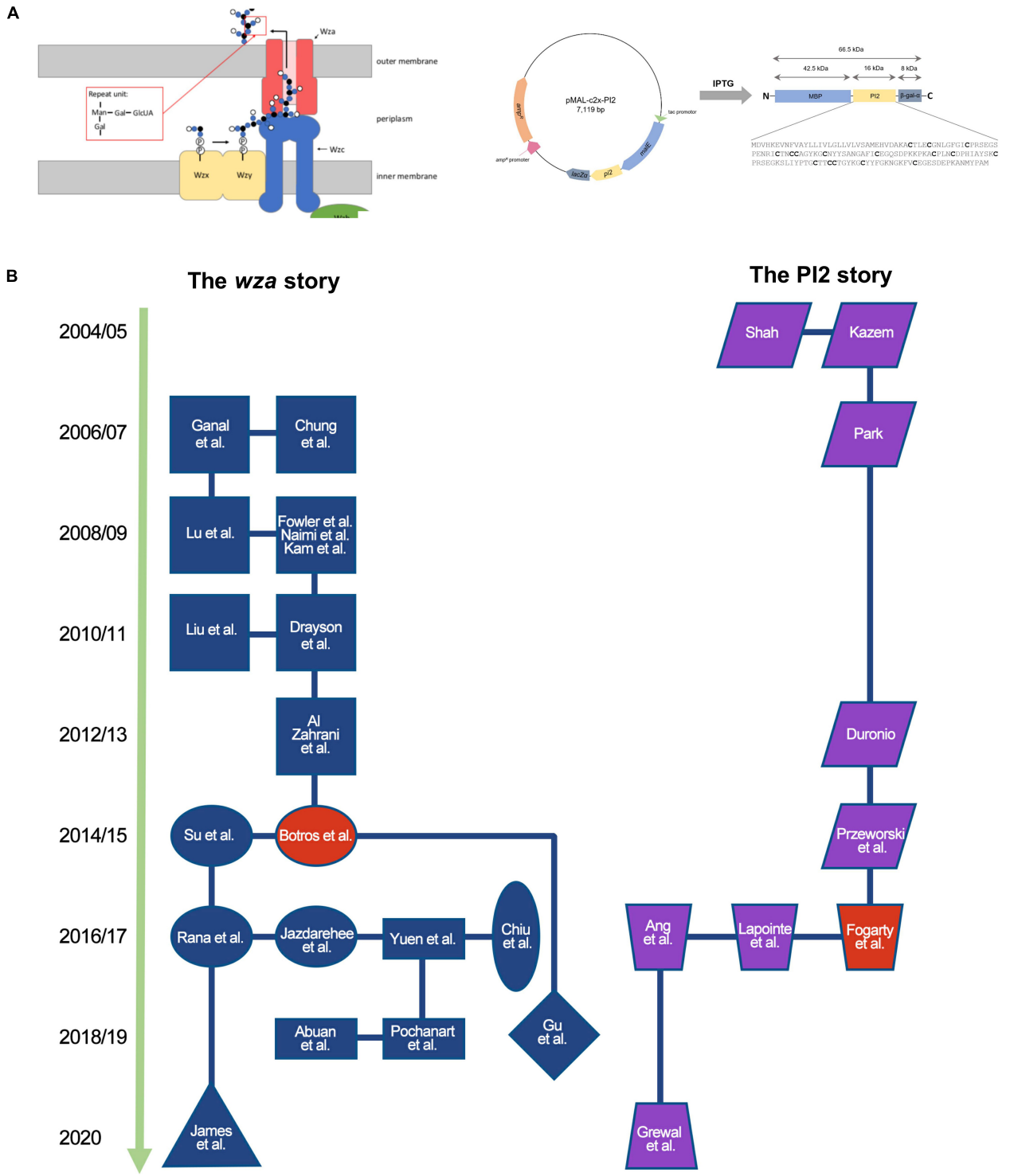

FIGURE 2 | UJEMI case studies demonstrate how each CURE build on each other over time. **(A)** Left: working model of proteins involved in the secretion of capsule in *E. coli* strain K30 (reprinted with permission, Yuen et al., 2017). Gene products discussed include Wza (red), Wzb (green), Wzc (blue). Capsule subunits are shown in small blue and black circles. Auxiliary secretion machinery subunits (Wzx, Wzy) are not discussed in the text. Right: Plasmid map, PI2-MBP fusion protein domain architecture, and primary amino acid sequence of PI2 (reprinted with permission, Grewal et al., 2020). Cysteine residues are shown in bold font. **(B)** Chronology of ongoing CURE-based research projects published in UJEMI investigating the capsule on antibiotic resistance (left, blue/red project nodes) and the expression and purification of protease inhibitor 2 (PI2) (right, purple/red project nodes). Projects are labeled with the name of the first author in the corresponding UJEMI publication (refer to **Supplementary Table S1**). Projects sharing similar research aims are depicted as the same shape project node. The red project nodes represent research articles describing key advancements in each project.

capsular polysaccharides and antibiotics may slow diffusion across the membrane (Slack and Nichols, 1982). Further, the regulation of capsule synthesis has been linked to stress response regulons in *E. coli* (Gottesman and Stout, 1991), leading to the notion that stress such as exposure to antibiotics may play a role in the regulation of capsule expression.

This project first began in our course when a student team decided to investigate the effects of sub-lethal doses of the antibiotics streptomycin and kanamycin on the synthesis of macromolecules in *E. coli* strain B23 (Chung et al., 2006). The students measured an increase in the concentration of hexose, a component of capsule, after treatment with the sub-lethal doses of the antibiotics (Chung et al., 2006). This study was followed up by two student teams who hypothesized that *E. coli* strain B23 treated with sub-lethal doses of kanamycin and streptomycin would increase capsule production (Ganal et al., 2007; Lu et al., 2008). The students found that capsule production increased following sublethal treatment with streptomycin and kanamycin (Ganal et al., 2007; Lu et al., 2008). However, follow-up studies were unable to link this phenotype with increased resistance to streptomycin (Fowler et al., 2009; Naimi et al., 2009), and increased resistance to kanamycin was observed in two studies (Kam et al., 2009; Al Zahrani et al., 2013), but not in a third (Drayson et al., 2011).

In 2014, the student team of Parmar et al. followed up on a report published in the journal Environmental Science and Pollution Research by researchers outside of the course suggesting that tetracycline interacts with capsular polysaccharides (Song et al., 2013). Parmar et al. asked whether capsule deficient mutants showed decreased resistance to tetracycline. The results of this study did not show a change in resistance to tetracycline (or streptomycin) in the capsular mutants (Parmar et al., 2014).

In 2014, the student team of Botros et al. initiated a new arm of the project by devising a screen to ask whether or not capsule contributes to resistance against a panel of 10 antibiotics representing different structural classes. The students drew upon the extensive research of Dr. Chris Whitfield at Guelph University in Canada whose lab has constructed defined deletion strains of the capsule secretion machinery in *E. coli* strain K30 (**Figure 2A** left; Whitfield, 2006). The students contacted Dr. Whitfield who generously provided wild type *E. coli* strain K30 and an isogenic strain (Δ*wza*−*wzb*−*wzc*) bearing a deletion of the genes encoding the outer membrane channel protein (Wza), the intermembrane ATPase (Wzb), and the inner membrane bound phosphatase (Wzc). Botros et al. developed a disk diffusion assay to semi-quantitatively compare the resistance of capsule deficient mutant strains and the wild type strain. After optimization, the disk diffusion assay was shown to be efficient and reliable. The results showed statistically significant differences in the zones of inhibition between the wild type and a capsule deficient mutant when treated with erythromycin and nitrofurantoin. Interestingly, resistance to erythromycin increased in the capsule deficient strain whereas resistance to nitrofurantoin decreased. Botros et al. chose to focus on the erythromycin result and followed up by showing that the phenotype was also observed for other macrolide antibiotics (e.g., clarithromycin, roxithromycin) but not for a ketolide (e.g., telithromycin). The student team concluded that deletion of the *E. coli* K30 group I capsule biosynthesis genes *wza, wzb,* and *wzc* confers capsule-independent resistance to macrolide antibiotics (Botros et al., 2015).

The next series of course projects utilized single gene deletion strains of Δ*wza,* Δ*wzb,* Δ*wzc* contributed again by Dr. Whitfield. After corroborating the results of Botros et al., student teams went on to show that deletion of *wza* is sufficient in conferring resistance to the macrolide erythromycin (Su et al., 2017) whereas deletion of *wzb* is not (Rana et al., 2016). Students also tested a Δ*wzc* deletion mutant which also showed a partially macrolide resistant phenotype (Jazdarehee et al., 2017).

The next set of student team projects asked whether complementation of *wza* in a strain bearing a deletion of this gene would restore the wild type (less erythromycin resistant) phenotype. The first attempt involved PCR amplification of the *wza* gene product and ligation into the TA TOPO cloning vector (Yuen et al., 2017). The student team of Yuen et al. designed PCR primers to amplify a product encompassing the putative *wza* promoter region to allow constitutive *wza* expression. The team obtained clones which they analyzed using Sanger sequencing. All of the inserts were found to be oriented in the same direction opposing the plasmid borne *lac* promoter sequence used for blue/white screening. The team surmised that the *wza* gene product may be lethal when overexpressed. Based on these results, the student team of Pochanart et al. decided to subclone the *wza* gene into a pBAD24 vector which encodes a promoter that can be upregulated and downregulated with the addition of media-based L-arabinose or glucose, respectively (Pochanart et al., 2018). The students were able to obtain clones which were verified by Sanger sequencing. Growth experiments showed that the high inducer concentration reduced the growth rate of the clones transformed with the *wza*-containing plasmid (Pochanart et al., 2018). This was consistent with the previous suggestion that overexpression of *wza* may be lethal (Yuen et al., 2017). The next student project set out to optimize the concentration of arabinose inducer to minimize the effect on growth rate (Abuan et al., 2018). After optimizing the inducer concentration, the students were able to show that in a strain bearing a deletion of *wza*, arabinose induction of a plasmid-encoded copy of *wza* was sufficient to restore erythromycin sensitivity of a Δ*wza* deletion strain using a disk diffusion assay (Abuan et al., 2018).

Students have started to explore the structure and function of the outer membrane channel protein Wza to understand how it is linked to the macrolide sensitivity. Using the crystal structure Wza (Dong et al., 2006), Su et al were able to measure the diameter, electrostatic properties and hydrophobicity of the pore. The students estimated the Wza pore to have a diameter of approximately 17 angstroms whereas the approximate size of erythromycin is 12 angstroms, suggesting that the channel may be sufficiently large enough to accommodate the antibiotic. The team acknowledged that electrostatic interactions and hydrophobicity of the Wza channel may also influence antibiotic movement through the channel. The student team of Chiu et al. (2017) followed up with a study that tested mutant specific tolerance to macrolides with different structural properties

including erythromycin, clarithromycin, and roxithromycin, and telithromycin. The authors reported that *wza* linked resistance was observed for erythromycin, clarithromycin, and roxithromycin but not for telithromycin, the latter having distinctive aromatic rings and ketone groups. Chiu et al. (2017) speculated that the additional ketone groups on telithromycin may increase its polarity which may influence how it crosses the membrane relative to the other tested macrolides. Surprisingly, a single *wza* deletion mutant was shown to be more resistant than a Δ*wza*−*wzb*−*wzc* triple deletion mutant when treated with azithromycin, perhaps insinuating a more complex, structure-specific model of antibiotic uptake (Chui et al., 2017).

The students have proposed a range of models to explain how deletion of Δ*wza* renders *E. coli* strain K30 resistant to macrolides. Su et al. (2017) suggested a model in which Wza stabilizes other outer membrane proteins involved in outer membrane integrity. Botros et al. suggested that the formation of K-LPS in the absence of the capsule secretion genes alter the stability of permeability of the outer membrane (personal communication with Dr. Chris Whitfield, 46). Finally, several studies on the macrolide resistant phenotype linked to *wza* have observed that the effect is limited to experiments done on solid media (disk diffusion assays) as opposed to liquid media (broth dilution assays) (Rana et al., 2016; Jazdarehee et al., 2017; Su et al., 2017). How the nature of the growth media influences the observed phenotype remains an open question. The student team of James et al. (2020) have asked whether the discrepancy between experiments performed in liquid vs. solid phase media reflect a phenotype related to biofilm formation (James et al., 2020). While a compelling hypothesis, James et al. reported that their data showed no correlation between biofilm production in liquid media and erythromycin resistance in *E. coli* K30 wild-type, Δ*wza*, and Δ*wza*−*wzb*−*wzc* (James et al., 2020).

A recent study by the student team of Gu et al. (2018) revisited the initial data describing the antibiotic screen published by Botros et al. (2015). Gu et al. (2018) were specifically interested in the observation that a triple deletion of Δ*wza*−*wzb*−*wzc* results in a decreased resistance to the antibiotic nitrofurantoin. Following an extensive effort to verify the DNA sequence of each of the mutations in each strain, the students showed that deletion of *wzb* is sufficient to decrease resistance to nitrofurantoin. To explain their data, Gu et al. (2018) present a working model in which nitrofurantoin toxicity is reduced in the absence of the *wzb* phosphatase, possibly by increasing the concentration of a phosphorylated form of a putative reductase.

THE PI2 STORY: PROTEIN EXPRESSION AND DISULPHIDE BOND FORMATION

The production of recombinant protein in a functionally folded conformation is a long-standing challenge faced by many microbiologists and biotechnologists (Rosano et al., 2019). The expression of proteins containing disulphide bonds in prokaryotic organisms such as *E. coli* is confounded by the naturally occurring net reducing redox state of the cytosol (Ren et al., 2016). Interested in better understanding the function of the reductase protein domain thioredoxin (Trx) that has been shown to promote the solubility of fusion proteins containing disulphide bonds, Shah (2004) initiated a study within our CURE to investigate the effect of a Trx fusion on solubility of proteinase inhibitor 2 (PI2) from potatoes. PI2 is a relatively small 21 kDa, dimeric, cysteine-rich, heat-stable, endo-acting peptidase that inhibits chymotrypsin and trypsin protein containing 16 cysteine residues predicted to form 8 disulphide bonds (Keil et al., 1986). Using a plasmid containing the PI2 gene sequence that was donated to the course, several iterative attempts were made at cloning the gene into the pET32 expression vector (Invitrogen) (Kazem, 2004; Shah, 2004; Park, 2006; Duronio, 2012; Przeworski et al., 2015). The student team of Geum et al. eventually constructed a PI2-Trx fusion plasmid that was confirmed by restriction enzyme analysis, however, overexpression of the PI2-Trx protein product was not observed in whole cell lysates of *E. coli* strain BL21(DE3) using SDS-PAGE analysis stained with Coomassie blue. Geum et al. tentatively concluded that pET32 and/or strain BL21(DE3) may not be a suitable expression vector/host for overexpression of PI2. In section "Future directions," the authors suggested Sanger sequencing to rule out mutations within their construct as well as Western blots as a more sensitive method of analysis (Geum et al., 2015).

In 2016, the student team of Fogarty et al. (2016) revisited the PI2 expression project. They began by using Sanger sequencing to determine the DNA sequence of the *pi2* insert and its genetic fusion to the thioredoxin domain (Fogarty et al., 2016). The authors analyzed the resulting DNA sequence to discover that the insert contained eukaryotic introns that resulted in a truncated protein due to an in-frame stop codon. The potato-derived *pi2* gene sequence also contained codons rarely used in *E. coli*. Fogarty et al. (2016) therefore adapted their project goal to design a version of the *pi2* sequence that lacked introns and was codon optimized for expression in *E. coli*. The team had their newly engineered DNA sequence synthesized as a gene block which they subcloned into a TOPO TA cloning plasmid. The next term, the student team of Lapointe et al. explored whether or not the newly designed *pi2* would be expressed when fused to either a maltose binding protein (MBP) domain or a hexahistidine tag (6XHis). The team subcloned the engineered *pi2* sequence from the TOPO TA plasmid construct into the commercially available pMALc2x and pET30b expression vectors that encode MBP and 6xHis tags, respectively (**Figure 2A** right). Expression analysis in BL21(DE3) transformed with each plasmid revealed a band in SDS-PAGE gels corresponding to the predicted molecular mass of PI2 fused to the MBP tag, although some protein degradation products were observed (Lapointe et al., 2016). Lapointe et al were the first to demonstrate PI2 expression and purification in our lab.

Ang et al. (2017) then opened a new branch of the project in our CURE by exploring whether or not altering the expression conditions or the cytosolic redox state of the *E. coli* expression host would impact PI2 expression levels. The authors compared PI2-MBP expression levels in *E. coli* strain Origami 2 (DE3) and *E. coli* wild type strain BL21(DE3). Origami 2 (DE3) bears mutations in glutaredoxin (*gor*) and thioredoxin (*trxB*) resulting in a net oxidizing cytoplasm. *E. coli* strain BL21 (DE3) encodes wild type copies of *gor* and *trxB* resulting in a net

reducing cytoplasm. Contrary to their hypothesis predicting higher expression levels of the cysteine rich PI2 in *E. coli* strain Origami 2, SDS PAGE analysis of whole cell lysates showed over-expressed protein corresponding with the molecular mass of PI2-MBP in BL21(DE3) but not in Origami 2 (DE3) (Ang et al., 2017).

In 2019, the student team of Grewal et al. followed up by attempting to express PI2-MBP in *E. coli* strain SHuffle (C3028), which has a net oxidative cytoplasm (Lobstein et al., 2012; Grewal et al., 2020). Unlike Origami 2, SHuffle expresses a disulfide bond isomerase, DsbC, that facilitates proper protein folding by disrupting the formation of non-native disulfide bonds (Grewal et al., 2020). SDS PAGE analysis revealed a band that corresponds to the expected molecular mass of PI2-MBP. Using maltose affinity chromatography, the students purified a soluble form of PI2-MBP. They probed the tertiary structure of the protein using limited proteolysis and observed distinct bands indicative of a uniformly folded protein structure as opposed to an irregular aggregated protein. The team recommended follow up studies to further assess folding and function of purified PI2-MBP.

DISCUSSION

These two case studies describe a series of authentic scientific research projects that build on each other over time. Carried out by undergraduate student teams pursuing hypothesis-driven questions as part of a CURE, each individual research project focuses on novel investigations and original ideas that contribute to working biological models (**Figure 2A**). The two case studies follow distinct branching patterns which are defined by the results of experimentation and curiosity driven research questions depicted as nodes in **Figure 2B**.

Consistent with the use of original research articles as the conventional approach to the dissemination of research results in science, UJEMI articles serve as concise records of a series of small student-driven research projects that provide literature-based linkages between projects within the course. This model has been an effective approach to CURE development for several reasons. First, similar to a maturing grant-funded research laboratory, the accumulation of reagents, and scientific knowledge increases the power and efficiency of the ongoing research projects, which is motivating to students as it has the potential to yield more frequent, impactful, and exciting discoveries. Second, by focusing on novel research questions the participants engage in dynamic projects with broad meaning and relevance. In fact, UJEMI articles have been cited in articles published by well-established professional research journals (Chang et al., 2010 cited in Burmeister et al., 2020). Third, the UJEMI literature-base creates a "community of practice." At the outset of the course, students are introduced to the journal as a repository of scientific investigations conducted by students who have come before them. Similar to any research project, they begin by "standing on the shoulders of giants" and they are expected to meet or exceed the effort and scientific rigor of their predecessors.

Each phase of the course uses UJEMI articles to facilitate student learning. In the planning phase, students read UJEMI papers, and derive new, follow-up research questions. In the experimentation phase, students experimentally verify the reliability of data in previous UJEMI papers looking for similarities and differences in results and interpretation before conducting novel analysis. In the dissemination phase, UJEMI articles are used as models for constructing a draft paper, as well as providing points for discussion. While the dissemination phase is notably short (i.e., 2–3 weeks to draft a manuscript), the students become familiar with the structure and function of UJEMI articles over the term before authoring their own manuscript. We surmise that by extensively working with the UJEMI articles in different contexts, the task of drafting a manuscript is made more efficient by indirectly scaffolding the writing phase with activities throughout the term that are linked to journal articles.

UJEMI articles provide students with concrete research topics and summaries of future directions, which enables student-driven project development by allowing the course instructor to provide arms-length verbal and written feedback to facilitate project development. In the planning phase, the instructor and teaching assistants provide written feedback on the individual proposal as well as the team proposal. The instructor and teaching assistants are also able to use team meetings to highlight aspects of previous studies that may impact the proposed research. Instructors often point out key papers in the field that the team should be aware of, known study limitations, and available research methods. The influence of the instructor on project development is more apparent in the dissemination phase when feedback is provided on the draft manuscript. Most often the instructor and teaching assistants work with the student authors to refine their paper in order to communicate evidence-based conclusions, clarify definitions, and explain ideas for future experiments that are both feasible and relevant. In cases where the research is communicated effectively in a UJEMI article, student teams tend to follow up with new research projects. If the research is communicated poorly, the projects tend to stall.

The prospect of being an author on a scientific manuscript is an aspect of our CURE model that promotes project ownership. Student authorship has previously been shown to benefit learning and research skill development in the context of CUREs (Cooper and Brownell, 2018; Corwin et al., 2018). All students participating in the course have the option of being included as an author on their team's manuscript. The default approach to authorship order is alphabetically by last name; however, in some instances, teams have decided to change the authorship order to acknowledge specific contributions. To change from the alphabetically ordered authorship, all team members must approve. The team-based nature of the course, and co-authorship on a UJEMI publication, also promotes a sense of collaborative ownership of the project. For example, we observe a trend in our student's written reflections about their research progress where they make statements moving from "...my project" to "...our project." We hypothesize that systematic analyses of student reflections written over the course of the term will be a valuable metric to measure positive shifts in student confidence, ownership and the value of collaboration in the scientific process.

Analysis of individual UJEMI papers provides evidence of practices consistent with the notion of scientific enculturation.

Case studies 1 and 2 include UJEMI articles that describe stages of scientific development in line with the outcomes of CURE participation predicted by Auchincloss et al. (2014). For example, writing the Introduction and Discussion sections requires content knowledge supported by credible scientific literature. The Methods and Materials section, as well as the Results section, capture a range of technical skill development, as well as collaboration skills as students share reagents within the course and interact with practicing scientists in the field. Since experimentation rarely follows a direct path, students learn to adapt their project goals and navigate uncertainty in their data. The conclusions sections of UJEMI papers reflect scientific maturity, as conclusions and claims are adjusted to more accurately reflect the data. Taken together, the process of doing authentic research through a journal-driven CURE means that students are fully immersed in the scientific experience. In order to meet the goal of publishing a scientific journal article, students need to engage with each of the CURE domains which comprehensively integrate the complex and dynamic processes underpinning the development of a scientist. A scientific project culminating in an original research article is an effective product to teach the process of doing science.

UJEMI articles are rich sources of objective data for understanding how our students are developing as scientists. A meta-analysis conducted by Linn et al. (2015) reported that more than half of 60 studies on undergraduate research experiences relied on subjective student-based, self-reporting surveys or interviews. The results of the study called for powerful and generalizable assessments to document student development to complement student surveys of perceived learning (Linn et al., 2015). Indeed, numerous studies have since described the development of validated and reliable survey instruments to assess student development in undergraduate research settings (Corwin et al., 2015; Shortlidge and Brownell, 2016; Ballen et al., 2017). Toward this end, we have collected preliminary data using the laboratory course assessment survey (LCAS) (Corwin et al., 2015) which showed student perceptions of learning aligned with the core domains of a CURE as outlined by Auchincloss et al. (2014). We are beginning to data mine and develop coding schemes for UJEMI articles to provide evidence of student learning within each domain of our CURE. One example is an assessment of scientific methodologies and skills developed as part of the CURE. Analysis of the Methods and Materials section of UJEMI articles provide evidence of techniques used by students in the course. Our preliminary data show that almost all student teams engage in *E. coli* strain isolation, PCR, Sanger Sequencing, and assay development/optimization. Since the projects are student-driven, the portfolio of techniques is not always predictable. Nevertheless, knowing what techniques are most commonly used helps the teaching team tailor scaffolding activities to guide student learning in the course. As a second example, evidence of collaboration can be gleaned from analyzing the Methods and Materials section as well as the Acknowledgments section of papers. Teams often recognize the contributions of other students in the course as well as researchers within and beyond the boundaries of our institution. Collaboration data informs course instructors of instances where scientific interactions can be fostered and better supported in future iterations of the course. We are also conducting deeper analyses of the writing assignments used to scaffold our CURE. Artifacts of learning, such as bi-weekly research summaries or research proposals, provide detailed accounts of activities including troubleshooting, reflection, and planning. We anticipate that additional meta-analyses of UJEMI papers, and associated writing assignments, will provide valuable integrated metrics of student development as scientists. Analyses over time will also provide dynamic perspectives reflecting the inner workings of our CURE to inform future curricula development to continually refine how best to meet the needs of our students.

The CURE described herein challenges students to develop and execute a novel research project with the goal of delivering a publication quality scientific manuscript in only 4 months. From the outset of the CURE, the students are made aware that their goal can be achieved by working as a team in a disciplined manner through a series of structured assignments that contribute to each research phase. A 2016 survey of alumni (n = 67) from our program showed that 93.9% of the respondents perceived their CURE experience as worthwhile, with 49 students (74.2%) indicating that the experience was "definitely" worthwhile and 13 students (19.7%) indicating that the experience was "somewhat" worthwhile. Three students (4.5%) indicated that the experience "had no value to them" and one student (1.5%) indicated that they felt the experience was "not a good use of their time." These survey data were supported by comments which included these reflections from two alumni:

"*Being able to do a project from scratch with so much freedom is something that I have not yet seen in any other course, but I feel is extremely important and helpful. In addition to the learning, the freedom was quite thrilling, it provided a feel of what science really is like (lots of time reading papers and troubleshooting), rather than sitting in a lecture theatre memorizing what a professor says, or following step by step procedures for an experiment I may not completely understand or care about how well the results for it turned out.*"

"*Overall, I think it was a really valuable experience, as all the other lab courses are basically cookbook style courses and here we were able to figure things out for ourselves and research what we were interested in. [. . .] it seemed a little daunting when starting the course, but it was really a lot of fun in the long run and I think I learned a lot.*"

These comments support the idea that student perceptions of learning align with the overall learning objectives of our CURE. Going forward we envision using mixed methods approaches combining validated survey instruments such as the LCAS, student reflections on learning, and coded analyses of learning artifacts such as UJEMI articles to better understand how CURE experiences can be designed and optimized to meet student needs.

CONCLUSION

Our journal-driven CURE model provides students with an opportunity to engage in a disciplined experience that guides

them through three critical phases of doing science: planning, experimentation, and dissemination. We depict these phases and their corresponding writing assignments as a cycle (**Figure 1**). Through iterative cycles of the course we have learned to appreciate the value of the time invested in each phase. A well-planned project with a testable hypothesis tends to provide concrete results and can be quite productive, especially in the context of a relatively short undergraduate course. The functional linkages between projects in the course underscore the value of the dissemination phase in terms of distilling information needed to carry on the project in another academic term. Further, the time constraint placed on the dissemination phase motivates students to summarize and communicate their findings in a timely manner. This model ostensibly reflects the process that most scientists would envision when taking on a new project; however, without structure, it is not uncommon for the planning and dissemination phases to be rushed or unbounded, respectively. Moreover, without formal milestones such as writing assignments, feedback critical for progressive development may be limited or absent altogether. We suggest that the research cycle model presented here may be useful, not only in CURE settings, but in other research settings in which trainees are developing including undergraduate internships or graduate studies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

DO and MG performed the conceptualization and acquisition of funding. DO prepared the original draft. All authors participated in the preparation and editing of this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.589025/full#supplementary-material

Supplementary Table 1 | Metadata compilation of UJEMI articles discussed in the two case studies.

REFERENCES

Abuan, K. A., AbuZuluf, H., Ban, Y., and Malekafzali, L. (2018). Plasmid-mediated complementation of *wza* restores erythromycin susceptibility in *Escherichia coli* K30 strain CWG28. *J. Exp. Microbiol. Immunol.* 4, 1–12.

Al Zahrani, F., Huang, M., Lam, B., and Vafaei, R. (2013). Capsule formation is necessary for kanamycin tolerance in *Escherichia coli* K12. *J. Exp. Microbiol. Immunol.* 17, 24–28.

Alford, R. F., Leaver-Fay, A., Gonzales, L., Dolan, E. L., and Gray, J. J. (2017). A cyber-linked undergraduate research experience in computational biomolecular structure prediction and design. *PLoS Comput. Biol.* 13:e1005837. doi: 10.1371/journal.pcbi.1005837

American Association for the Advancement of Science, (2011). *Vision and Change in Undergraduate Biology Education: A Call to Action*. Washington, DC: American Association for the Advancement of Science.

Ang, I., Atte, A., Halim, B., and Jassal, J. (2017). Effect of temperature, inducer concentration, and *escherichia coli* cytosolic redox state on mbp-pi2 expression. *J. Exp. Microbiol. Immunol.* 21, 11–14.

Auchincloss, L. C., Laursen, S. L., Branchaw, J. L., Eagan, K., Graham, M., Hanauer, D. I., et al. (2014). Assessment of course-based undergraduate research experiences: a meeting report. *CBE Life Sci. Educ.* 13, 29–40. doi: 10.1187/cbe.14-01-0004

Ayella, A., and Beck, M. R. (2018). A course-based undergraduate research experience investigating the consequences of nonconserved mutations in lactate dehydrogenase. *Biochem. Mol. Biol. Educ. Bimon. Publ. Int. Union Biochem. Mol. Biol.* 46, 285–296. doi: 10.1002/bmb.21115

Backstrom, I., Johnson, I. H., Lien, S., and Ragotte, R. (2017). cpxP deletion confers resistance to misfolded papeinduced cytotoxicity through enhanced CpxAR activation in *Escherichia coli*. *J. Exp. Microbiol. Immunol.* 3, 10–16.

Ballen, C. J., Blum, J. E., Brownell, S., Hebert, S., Hewlett, J., Klein, J. R., et al. (2017). A call to develop course-based undergraduate research experiences (CUREs) for nonmajors courses. *CBE Life Sci. Educ.* 16:mr2. doi: 10.1187/cbe.16-12-0352

Bangera, G., and Brownell, S. E. (2014). Course-based undergraduate research experiences can make scientific research more inclusive. *CBE Life Sci. Educ.* 13, 602–606. doi: 10.1187/cbe.14-06-0099

Beck, C., Butler, A., Burke, and da Silva, K. (2014). Promoting inquiry-based teaching in laboratory courses: are we meeting the grade? *CBE—Life Sci. Educ.* 13, 444–452. doi: 10.1187/cbe.13-12-0245

Bhattacharyya, P., Chan, C. W. M., Duchesne, R. R., Ghosh, A., Girard, S. N., and Ralston, J. J. (2020). Course-based research: a vehicle for broadening access to undergraduate research in the twenty-first century - proquest. *SPUR* 3, 14–27. doi: 10.18833/spur/3/3/7

Botros, S., Mitchell, D., and Ommen, C. V. (2015). Deletion of the *Escherichia coli* K30 group I capsule biosynthesis genes *wza, wzb and wzc* confers capsule-independent resistance to macrolide antibiotics. *J. Exp. Microbiol. Immunol.* 19, 1–8.

Brownell, S. E., Hekmat-Scafe, D. S., Singla, V., Chandler Seawell, P., Conklin Imam, J. F., Eddy, S. L., et al. (2015). A high-enrollment course-based undergraduate research experience improves student conceptions of scientific thinking and ability to interpret data. *CBE Life Sci Educ.* 14:ar21. doi: 10.1187/cbe.14-05-0092

Burmeister, A. R., Fortier, A., Roush, C., Lessing, A. J., Bender, R. G., Barahman, R., et al. (2020). Pleiotropy complicates a trade-off between phage resistance and antibiotic resistance. *Proc. Natl. Acad. Sci. U.S.A.* 117, 11207–11216. doi: 10.1073/pnas.1919888117

Chang, V., Chen, L.-Y., Wang, A., and Yuan, X. (2010). The effect of lipopolysaccharide core structure defects on transformation efficiency in isogenic *Escherichia coli* BW25113 *rfag, rfap*, and *rfac* mutants. *J. Exp. Microbiol. Immunol.* 14, 101–107.

Cheng, M., Remedios, R. D., Lu, L., and Tang, C. (2019). *Lactobacillus rhamnosus* GG does not increase *Caenorhabditis elegans* longevity. *J. Exp. Microbiol. Immunol.* 24, 1–9.

Chiu, J., Han, G., McCrystal, K., and Zuo, M. (2017). Macrolide structures can confer differential susceptibility in *Escherichia coli* K30 deletions of group 1 capsule assembly genes. *J. Exp. Microbiol. Immunol.* 3, 50–56.

Chui, J., Croft, C., and Ng, K. (2017). *Escherichia coli* O antigen serotype O16 is a restriction factor for bacteriophage T4 infection. *J. Exp. Microbiol. Immunol.* 3, 38–44.

Chung, C., Hung, G., Lam, C., and Madera, L. (2006). Secondary effects of streptomycin and kanamycin on macromolecular composition of *Escherichia coli* B23 cell. *J. Exp. Microbiol. Immunol.* 9, 11–15.

Coil, D., Wenderoth, M. P., Cunningham, M., and Dirks, C. (2010). Teaching the process of science: faculty perceptions and an effective methodology. *CBE Life Sci. Educ.* 9, 524–535. doi: 10.1187/cbe.10-01-0005

Cooper, K. M., Blattman, J. N., Hendrix, T., and Brownell, S. E. (2019). The impact of broadly relevant novel discoveries on student project ownership in a traditional lab course turned CURE. *CBE Life Sci. Educ.* 18:ar57. doi: 10.1187/cbe.19-06-0113

Cooper, K. M., and Brownell, S. E. (2018). Developing course-based research experiences in discipline-based education research: lessons learned and recommendations. *J. Microbiol. Biol. Educ.* 19:jmbe-19-88.

Corwin, L. A., Runyon, C., Robinson, A., and Dolan, E. L. (2015). The laboratory course assessment survey: a tool to measure three dimensions of research-course design. *CBE—Life Sci. Educ.* 14:ar37. doi: 10.1187/cbe.15-03-0073

Corwin, L. A., Runyon, C. R., Ghanem, E., Sandy, M., Clark, G., Palmer, G. C., et al. (2018). Effects of discovery, iteration, and collaboration in laboratory courses on undergraduates' research career intentions fully mediated by student ownership. *CBE Life Sci. Educ.* 17:ar20. doi: 10.1187/cbe.17-07-0141

Cramb, K., Bakkeren, E., Rafaeli, I., and Oliver, D. (2015). The zinc ion-chelating agent TPEN reduces CpxP-mediated negative regulation of 17 the CpxAR two-component system in *Escherichia coli*. *J. Exp. Microbiol. Immunol.* 1, 1–8.

Dimou, J., Lu, J., Pflueger, S., and Toombs, E. (2019). Rescue of O16 antigen expression in *E. coli strain MG*1655 prevents adsorption of T4 bacteriophage. *J. Exp. Microbiol. Immunol.* 5, 1–7.

Dong, C., Beis, K., Nesper, J., Brunkan-Lamontagne, A. L., Clarke, B. R., Whitfield, C., et al. (2006). Wza the translocon for *E. coli* capsular polysaccharides defines a new class of membrane protein. *Nature* 444, 226–229. doi: 10.1038/nature05267

Drayson, R., Leggat, T., and Wood, M. (2011). Increased antibiotic resistance post-exposure to sub-inhibitory concentrations is independent of capsular polysaccharide production in *Escherichia coli*. *J. Exp. Microbiol. Immunol.* 15, 36–42.

Duronio, C. (2012). Production of a recombinant vector to enable the study of thioredoxin function as a bound or detached solubilizer of proteinase inhibitor 2 in a bacterial protein overexpression system. *J. Exp. Microbiol. Immunol.* 16, 79–84.

Esparza, D., Wagler, A. E., and Olimpo, J. T. (2020). Characterization of instructor and student behaviors in CURE and Non-CURE learning environments: impacts on student motivation, science identity development, and perceptions of the laboratory experience. *CBE Life Sci. Educ.* 19:ar10. doi: 10.1187/cbe.19-04-0082

Estrada, M., Hernandez, P. R., and Schultz, P. W. (2018). A longitudinal study of how quality mentorship and research experience integrate underrepresented minorities into STEM careers. *CBE Life Sci. Educ.* 17:ar9. doi: 10.1187/cbe.17-04-0066

Feldman, A., Divoll, K. A., and Rogan-Klyve, A. (2013). Becoming researchers: the participation of undergraduate and graduate students in scientific research groups. *Sci. Educ.* 97, 218–243. doi: 10.1002/sce.21051

Florence, M. K., and Yore, L. D. (2004). Learning to write like a scientist: coauthoring as an enculturation task. *J. Res. Sci. Teach.* 41, 637–668. doi: 10.1002/tea.20015

Fogarty, E., Alimohammadi, A., Siu, J., and Stachowiak, A. (2016). Synthesis, cloning, and sequencing of a codon optimized variant of proteinase inhibitor ii designed for expression in *Escherichia coli*. *J. Exp. Microbiol. Immunol.* 20, 100–105.

Fowler, D., Hu, J., Hou, P., and Wong, C. (2009). The effect of sub-inhibitory streptomycin on capsular polysaccharide production and streptomycin resistance in *Escherichia coli*. *J. Exp. Microbiol. Immunol.* 13, 47–52.

Ganal, S., Gaudin, C., Roensch, K., and Tran, M. (2007). Effects of streptomycin and kanamycin on the production of capsular polysaccharides in *Escherichia coli* B23 cells. *J. Exp. Microbiol. Immunol.* 11, 54–59.

Geum, L., Huber, R., Leung, N., and Lowe, M. (2015). Construction of recombinant expression vectors to study the effect of thioredoxin on heterologous protein solubility. *J. Exp. Microbiol. Immunol.* 19, 1–5.

Goldhawke, B., Kalhon, M., Lotto, J., and Deeg, C. M. (2016). Yeasts from greenhouse grapes show less phenotypic and genetic diversity than yeasts from vineyard grapes when isolated from grape crush cultured in liquid media. *J. Exp. Microbiol. Immunol.* 2, 8–15.

Gottesman, S., and Stout, V. (1991). Regulation of capsular polysaccharide synthesis in *Escherichia coli* K12. *Mol. Microbiol.* 5, 1599–1606. doi: 10.1111/j.1365-2958.1991.tb01906.x

Grewal, R., Kim, W., Shi, D., and Tong, H. (2020). Comparative expression of potato proteinase 1 inhibitor type II in an oxidative 2 versus reductive cytosolic environment of *Escherichia coli*. 6, 1–10.

Gu, M., Khan, A., Pagulayan, D. S., and Tam, W. L. (2018). Deletion of the capsule phosphatase gene *wzb* renders *Escherichia coli* strain K30 sensitive to the antibiotic nitrofurantoin. *J. Exp. Microbiol. Immunol.* 4, 1–15.

Hartstein, S., Kim, C., Phan, K., Windt, D., and Oliver, D. C. (2017). *Escherichia coli* OmpC mutants are sensitive to ethylenediaminetetraacetic acid and sodium dodecyl sulfate treatment whereas double OmpC and OmpF mutants are not. *J. Exp. Microbiol. Immunol.* 3, 17–21.

James, C., Kim, C., Pan, C., and Zhong, D. (2020). Biofilm production in *Escherichia coli* K30 with group 1 capsular gene *wza* and *wza-wzb-wzc* deletions is not correlated with erythromycin resistance phenotypes in liquid media. *J. Exp. Microbiol. Immunol.* 25, 1–9.

James, C. G., Morah, O., Panwal, V., and Yarmand, A. (2018). Survival of *Caenorhabditis elegans* infected with *Escherichia coli* DFB1655 is not affected by a missense mutation in *dop-1* or treatment with chlorpromazine hydrochloride. *J. Exp. Microbiol. Immunol.* 4, 1–9.

Jazdarehee, A., Anderson, J. J., Morrison, D., and Pardoe, W. (2017). Deletion of *Escherichia coli* K30 type I capsule assembly gene wzc confers resistance to the antibiotic erythromycin in solid media. *J. Exp. Microbiol. Immunol.* 21, 108–112.

Kam, J., Luo, X. L., and Song, H. A. (2009). Effects of reduced capsular polysaccharide on kanamycin resistance in *Escherichia coli* B23 cells. *J. Exp. Microbiol. Immunol.* 13, 22–28.

Kazem, M. (2004). Cloning EDTA monooxygenase as a model protein to characterize the effects of flavin oxidoreductase on solubility of proteins. *J. Exp. Microbiol. Immunol.* 6, 26–34.

Keil, M., Sanchez-Serrano, J., Schell, J., and Willmitzer, L. (1986). Primary structure of a proteinase inhibitor II gene from potato (*Solanum tuberosum*). *Nucleic Acids Res.* 14, 5641–5650. doi: 10.1093/nar/14.14.5641

Kerr, M. A., and Yan, F. (2016). Incorporating course-based undergraduate research experiences into analytical chemistry laboratory curricula. *J. Chem. Educ.* 93, 658–662. doi: 10.1021/acs.jchemed.5b00547

Lapointe, H. R., Li, S., Mortazavi, S., and Zeng, J. (2016). Expression and purification of a potato type II proteinase inhibitor in *Escherichia coli strain* BL21(DE3). *J. Exp. Microbiol. Immunol.* 2, 34–40.

Laursen, S., Hunter, A.-B., Seymour, E., Thiry, H., and Melton, G. (2010). *Undergraduate Research in the Sciences: Engaging Students in Real Science*. Hoboken, NJ: John Wiley & Sons, 314.

Light, C., Fegley, M., and Stamp, N. (2019). Role of research educator in sequential course-based undergraduate research experience program. *FEMS Microbiol. Lett.* 366:fnz140.

Linn, M. C., Palmer, E., Baranger, A., Gerard, E., and Stone, E. (2015). Undergraduate research experiences: impacts and opportunities. *Science* 347:1261757. doi: 10.1126/science.1261757

Lobstein, J., Emrich, C. A., Jeans, C., Faulkner, M., Riggs, P., and Berkmen, M. (2012). SHuffle, a novel *Escherichia coli* protein expression strain capable of correctly folding disulfide bonded proteins in its cytoplasm. *Microb. Cell Fact.* 11:56.

Lopatto, D. (2004). Survey of Undergraduate Research Experiences (SURE): first findings. *Cell Biol. Educ.* 3, 270–277. doi: 10.1187/cbe.04-07-0045

Lopatto, D. (2010). Undergraduate research as a high-impact student experience. *Peer Rev.* 12, 27–30.

Lu, E., Trinh, T., Tsang, T., and Yeung, J. (2008). Effect of growth in sublethal levels of kanamycin and streptomycin on capsular polysaccharide production and antibiotic resistance in *Escherichia coli* B23. *J. Exp. Microbiol. Immunol.* 12, 21–26.

Naimi, I., Nazer, M., Ong, L., and Thong, E. (2009). The role of *wza* in extracellular capsular polysaccharide levels during exposure to sublethal doses of streptomycin. *J. Exp. Microbiol. Immunol.* 13, 36–40.

Olimpo, J. T., Fisher, G. R., and DeChenne-Peters, S. E. (2016). Development and evaluation of the tigriopus course-based undergraduate research experience: impacts on students' content knowledge, attitudes, and motivation in a majors introductory biology course. *CBE Life Sci. Educ.* 15:ar72. doi: 10.1187/cbe.15-11-0228

Park, J. E. (2006). Generation of recombinant plasmid constructs to assess the ability of NADH:flavin oxidoreductase to solubilize proteinase inhibitor 2 in bacterial protein overexpression systems. *J. Exp. Microbiol. Immunol.* 10, 27–33.

Parmar, S., Rajwani, A., Sekhon, S., and Suri, K. (2014). The *Escherichia coli* K12 capsule does not confer resistance to either tetracycline or streptomycin. *J. Exp. Microbiol. Immunol.* 18, 76–81.

Pochanart, A., Richardson, M., Truong, P., and Wang, J. (2018). Plasmid mediated complementation of *wza* in *Escherichia coli* K30 strain CWG281 restores erythromycin sensitivity. *J. Exp. Microbiol. Immunol.* 22, 1–14.

Przeworski, C., Pham, D., Wang, I., and Murillo, J. (2015). Attempted construction of recombinant vectors designed to study the solubility of overexpressed proteinase inhibitor 2 when co-expressed with thioredoxin. *J. Exp. Microbiol. Immunol.* 19, 1–6.

Rana, G., Jang, Y., Ahn, P., and Nan, J. (2016). Single deletion of *Escherichia coli* K30 group i capsule biosynthesis system component, *wzb*, is not sufficient to confer capsule-independent resistance to erythromycin. *J. Exp. Microbiol. Immunol.* 20, 19–24.

Ren, G., Ke, N., and Berkmen, M. (2016). Use of the SHuffle strains in production of proteins. *Curr. Protoc. Protein Sci.* 85, 5.26.1–5.26.21.

Robnett, R. D., Chemers, M. M., and Zurbriggen, E. L. (2015). Longitudinal associations among undergraduates' research experience, self-efficacy, and identity. *J. Res. Sci. Teach.* 52, 847–867. doi: 10.1002/tea.21221

Rosano, G. L., Morales, E. S., and Ceccarelli, E. A. (2019). New tools for recombinant protein production in *Escherichia coli*: a 5-year update. *Protein Sci.* 28, 1412–1422. doi: 10.1002/pro.3668

Russell, S. H., Hancock, M. P., and McCullough, J. (2007). Benefits of undergraduate research experiences. *Science* 316, 548–549. doi: 10.1126/science.1140384

Sadler, T. D., Burgin, S., McKinney, L., and Ponjuan, L. (2010). Learning science through research apprenticeships: a critical review of the literature. *J. Res. Sci. Teach.* 47, 235–256.

Sarmah, S., Chism, G. W., Vaughan, M. A., Muralidharan, P., Marrs, J. A., and Marrs, K. A. (2016). Using zebrafish to implement a course-based undergraduate research experience to study teratogenesis in two biology laboratory courses. *Zebrafish* 13, 293–304. doi: 10.1089/zeb.2015.1107

Seymour, E., Hunter, A.-B., Laursen, S. L., and DeAntoni, T. (2004). Establishing the benefits of research experiences for undergraduates in the sciences: first findings from a three-year study. *Sci. Educ.* 88, 493–534. doi: 10.1002/sce.10131

Shah, N. (2004). Preparing plasmid constructs to investigate the characteristics of thiol reductase and flavin reductase with regard to solubilizing insoluble proteinase inhibitor 2 in bacterial protein overexpression systems. *J. Exp. Microbiol. Immunol.* 6, 20–25.

Shanle, E. K., Tsun, I. K., and Strahl, B. D. (2016). A course-based undergraduate research experience investigating p300 bromodomain mutations. *Biochem. Mol. Biol. Educ. Bimon. Publ. Int. Union Biochem. Mol. Biol.* 44, 68–74. doi: 10.1002/bmb.20927

Shapiro, C., Moberg-Parker, J., Toma, S., Ayon, C., Zimmerman, H., Roth-Johnson, E. A., et al. (2015). Comparing the impact of course-based and apprentice-based research experiences in a life science laboratory curriculum. *J. Microbiol. Biol. Educ.* 16, 186–197. doi: 10.1128/jmbe.v16i2.1045

Shelby, S. J. (2019). A course-based undergraduate research experience in biochemistry that is suitable for students with various levels of preparedness. *Biochem. Mol. Biol. Educ.* 47, 220–227. doi: 10.1002/bmb.21227

Shortlidge, E. E., and Brownell, S. E. (2016). How to assess your CURE: a practical guide for instructors of course-based undergraduate research experiences. *J. Microbiol. Biol. Educ.* 17, 399–408. doi: 10.1128/jmbe.v17i3.1103

Slack, M., and Nichols, W. (1982). Antibiotic penetration through bacterial capsules and exopolysaccharides. *J. Antimicrob. Chemother.* 10, 368–372. doi: 10.1093/jac/10.5.368

Song, C., Sun, X.-F., Xing, S.-F., Xia, P.-F., Shi, Y., and Wang, S.-G. (2013). Characterization of the interactions between tetracycline antibiotics and microbial extracellular polymeric substances with spectroscopic approaches. *Environ. Sci. Pollut. Res. Int.* 21, 1786–1795. doi: 10.1007/s11356-013-2070-6

Stoeckman, A., Cai, Y., and Chapman, K. (2019). iCURE (iterative course-based undergraduate research experience): a case-study. *Biochem. Mol. Biol. Educ.* 47, 565–572. doi: 10.1002/bmb.21279

Su, A. M., Wang, A., and Yeo, L. (2017). Deletion of the group 1 capsular gene wza in *Escherichia coli* E69 confers resistance to the antibiotic erythromycin on solid media but not in liquid media. *J. Exp. Microbiol. Immunol.* 3, 1–8.

Sun, E., Huggins, J. A., Brown, K. L., Boutin, R. C. T., Ramey, W. D., Graves, M. L., et al. (2020). Development of a peer-reviewed open-access undergraduate research journal. *J. Microbiol. Biol. Educ.* 21, 21.2.62.

The University of British Columbia, (2020). *Overview and Facts*. Vancouver, BC: The University of British Columbia.

Weaver, G. C., Russell, C. B., and Wink, D. J. (2008). Inquiry-based and research-based laboratory pedagogies in undergraduate science. *Nat. Chem. Biol.* 4, 577–580. doi: 10.1038/nchembio1008-577

Whitfield, C. (2006). Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*. *Annu. Rev. Biochem.* 75, 39–68. doi: 10.1146/annurev.biochem.75.103004.142545

Wolkow, T. D., Jenkins, J., Durrenberger, L., Swanson-Hoyle, K., and Hines, L. M. (2019). One early course-based undergraduate research experience produces sustainable knowledge gains, but only transient perception gains. *J. Microbiol. Biol. Educ.* 20:20.2.32.

Yuen, B., Ting, J., Kang, K., and Wong, T. (2017). Investigation of Wza in erythromycin sensitivity of *Escherichia coli* K30 E69 by genetic complementation. *J. Exp. Microbiol. Immunol.* 21, 52–57.

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PERSPECTIVE
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The Human Microbiome as a Focus of Antibiotic Discovery: *Neisseria mucosa* Displays Activity Against *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae infections are a serious global health problem. This organism has developed disturbing levels of antibiotic resistance, resulting in the need for new approaches to prevent and treat gonorrhea. The genus *Neisseria* also includes several members of the human microbiome that live in close association with an array of microbial partners in a variety of niches. We designed an undergraduate antibiotic discovery project to examine a panel of nonpathogenic *Neisseria* species for their ability to produce antimicrobial secondary metabolites. Five strains belonging to the *N. mucosa* species group displayed activity against other *Neisseria* in delayed antagonism assays; three of these were active against *N. gonorrhoeae*. The antimicrobial compound secreted by *N. mucosa* NRL 9300 remained active in the presence of catalase, trypsin, and HEPES buffer, and effectively inhibited a DNA uptake mutant of *N. gonorrhoeae*. Antimicrobial activity was also retained in an ethyl acetate extract of plate grown *N. mucosa* NRL 9300. These data suggest *N. mucosa* produces an antimicrobial secondary metabolite that is distinct from previously described antigonococcal agents. This work also serves as a demonstration project that could easily be adapted to studying other members of the human microbiome in undergraduate settings. We offer the perspective that both introductory and more advanced course-based and apprentice-style antibiotic discovery projects focused on the microbiome have the potential to enrich undergraduate curricula and we describe transferrable techniques and strategies to facilitate project design.

Keywords: human microbiome, antibiotic discovery, undergraduate research, antibiotic resistance, nonpathogenic *Neisseria* species, *Neisseria mucosa*, gonorrhea, secondary metabolites

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INTRODUCTION

Antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* represents a major public health concern. This human-restricted Gram negative diplococcus is responsible for approximately 87 million cases of gonorrhea per year globally (Rowley et al., 2019). Sexual transmission of *N. gonorrhoeae* can result in infection of the female and male urogenital tract, rectum, and pharynx. In addition, infected women can transmit the organism during delivery, resulting in neonatal conjunctivitis. Treatment options are generally limited to dual therapy with two antibiotics (ceftriaxone plus azithromycin), and gonococcal strains exhibiting resistance to all recommended antimicrobial

agents have recently been identified (Eyre et al., 2018; Whiley et al., 2018; Unemo et al., 2019). This pressing global problem has prompted the US Centers for Disease Control and Prevention (2012), World Health Organization, Department of Reproductive Health and Research (2012), and European Centre for Disease Prevention and Control (2019) to develop public health response plans addressing *N. gonorrhoeae* AMR, and new approaches for the prevention and treatment of gonococcal infections are desperately needed.

Neisseria species include *N. gonorrhoeae*, *N. meningitidis*, which can asymptomatically colonize the human nasopharynx and also cause deadly meningococcal meningitis, several nonpathogenic species that are components of the normal human oropharyngeal microbiome, and nonhuman isolates (Liu et al., 2015). The genus *Neisseria* is among the ten most abundant genera in the human microbiome (Human Microbiome Project Consortium, 2012), and co-colonization and horizontal gene transfer involving pathogenic and nonpathogenic *Neisseria* are well documented (e.g., Spratt et al., 1992; Maiden, 1998; Marri et al., 2010; Weyand, 2017). The nonpathogenic *Neisseria* are of particular interest because natural products produced by members of the human microbiome may represent a source of novel antimicrobial agents (Donia and Fischbach, 2015; Wilson et al., 2017; Milshteyn et al., 2018). For example, the commensal organism *Staphylococcus lugdunensis* found in the human nasal microbiome produces the secondary metabolite lugdunin, which displays antimicrobial activity against AMR *S. aureus* (Zipperer et al., 2016). Little is known about production of secondary metabolites by *Neisseria*, however, some nonpathogenic species have been shown to inhibit pathogens. *N. lactamica* (Deasy et al., 2015) and *N. cinerea* (Custodio et al., 2020) inhibit the colonization of *N. meningitidis* in human infection and epithelial cell culture models, respectively, and DNA released by *N. elongata* and multiple other nonpathogenic *Neisseria* species kills *N. gonorrhoeae* (Kim et al., 2019). In the project described here, we initiated a search for neisserial antimicrobial secondary metabolites by analyzing a panel of 36 nonpathogenic *Neisseria* strains for activity against other *Neisseria*, including *N. gonorrhoeae.* We identified five active isolates, all from the *N. mucosa* species group, and used inhibitor studies and chemical extraction to characterize the activity displayed by *N. mucosa* strain 9300.

This work was accomplished in a liberal arts college setting utilizing both traditional apprentice-style and course-based undergraduate research approaches that included both introductory and more advanced students. Projects focusing on AMR and antibiotic discovery have proven to be particularly effective at involving undergraduate students in meaningful research, connecting them to important social issues, and enlivening their study of various aspects of microbiology (e.g., Hoskisson et al., 2015; Smyth, 2017; Dube, 2018; Genné-Bacon and Bascom-Slack, 2018; Hernandez et al., 2018). Many prior projects focused on searching for antibiotic producing bacteria in environmental samples. For example, Tiny Earth[1] represents a highly successful initiative in which students analyze soil for the presence of bacteria that display inhibitory activity against a panel of target strains, or "safe relatives," closely related to a set of pathogens commonly responsible for nosocomial infections displaying AMR (Hernandez et al., 2018). Here, we offer the perspective the human microbiome represents another ecosystem that is ripe for undergraduate antibiotic discovery projects. We describe tools for screening nonpathogenic members of the microbiome for antimicrobial activity and offer a multiphasic project design for engaging different populations of undergraduate students in traditional and course-based research as a project evolves over time. The strategies, methods, and results described below demonstrate the scientific promise of this approach and offer an undergraduate research model that might be adapted and expanded by others interested in microbiome-based natural products discovery.

[1]https://tinyearth.wisc.edu/

MATERIALS AND METHODS

Bacterial Isolates, Growth Conditions, and Species Confirmation by *rplF* Sequence Analysis

Table 1 lists the nonpathogenic *Neisseria* strains used in this study. *Neisseria* were grown on GCB agar (Difco Laboratories) containing Kellogg's supplements I and II (Kellogg et al., 1963) and incubated at 37°C with 5% CO_2. Isolate identity for NRL strains was confirmed by PCR amplification and sequencing of a 413 bp fragment of the *rplF* gene followed by analysis using the PubMLST *Neisseria* database as previously described (Bennett et al., 2014; Jolley et al., 2018). The *rplF* sequences generated in this study were deposited in GenBank under the accession numbers given in **Table 1**. Strains were assigned to species groups as proposed by Bennett et al. (2012). The following well-established laboratory strains of *N. gonorrhoeae* were used as targets to test for antibiotic activity: ATCC 19424, ATCC 43070, FA19, F62, MS11, FA1090, and FA6140.

Screening for Antimicrobial Activity

Delayed antagonism cross-streak assays were used to test 24-h cultures of nonpathogenic *Neisseria* for their ability to produce antimicrobial substances (**Figure 1**). A linear streak of each potential producer isolate was made down the center of a GCB agar plate. Plates were incubated for 48 h and target strains were then streaked at right angles to the line of original growth. Plates were examined after 24 h of additional incubation for inhibition of target strain growth in the vicinity of the central streak. A strain was considered positive for antimicrobial activity against the target if a clear zone greater than 0.5 mm was present where the target strain had been inhibited (n = at least three trials per strain for each target).

A confirmatory agar overlay assay was performed on all *Neisseria* strains that displayed antimicrobial activity in the initial cross streak screening. PBS (Amresco) suspensions containing 10^9 CFU/ml of producer strains were prepared from 24 h plate cultures and 10 μl samples of the suspensions were spotted

TABLE 1 | Inhibitory activity of nonpathogenic *Neisseria* in delayed antagonism assays.

	Target Strains											
	N. lactamica		***N. flavescens***		***N. gonorrhoeae***		**Additional *N. gonorrhoeae* strains[4]**					
	ATCC 23970		**N47**		**ATCC43070**		**FA1090**	**FA19**	**F62**	**19424**	**MS11**	**FA6140**
Nonpathogenic *Neisseria* species[1,2,3]	**CS**	**AO**	**CS**	**AO**	**CS**	**AO**	**AO**	**AO**	**AO**	**AO**	**AO**	**AO**
***N. mucosa* group**												
N. mucosa NRL 9300 [MW166311]	+	8	+	15	+	11	10	10	9	10	11	10
N. mucosa NRL 9297 [MW166312]	–	–	+	12	+	10						
N. mucosa ATCC 25996	–	–	+	14	+	10						
N. mucosa ATCC 19696	–	–	–	–	–	–						
N. sicca NRL 30016 [MW166313]	–	–	+	9	–	–						
N. sicca ATCC 29256	–	–	+	11	–	–						
N. sicca ATCC 9913	–	–	–	–	–	–						
N. sicca NRL 272 [MW166314]	–	–	–	–	–	–						
***N. cinerea* group**	–		–		–							
N. cinerea ATCC 14685	–		–		–							
N. cinerea NRL 32828 [MW160215]	–		–		–							
N. cinerea NRL 33295 [MW160216]	–		–		–							
N. cinerea NRL 33683 [MW166303]	–		–		–							
N. cinerea NRL 33807 [MW166304]	–		–		–							
***N. lactamica* group**												
N. lactamica ATCC 23970	–		–		–							
N. lactamica NRL 36016 [MW166305]	–		–		–							
N. lactamica NRL 36046 [MW166306]	–		–		–							
N. lactamica NRL 36121 [MW166308]	–		–		–							
N. lactamica NRL 37168 [MW166309]	–		–		–							
N. lactamica NRL 37170 [MW166310]	–		–		–							
N. lactamica NRL37174 [MW166307]	–		–		–							
***N. subflava* group**												
N. flava NRL 30008 [MW166315]	–		–		–							
N. flava NRL 30037 [MW166316]	–		–		–							
N. flava NRL 9994 [MW166317]	–		–		–							
N. flava NRL 9298 [MW166318]	–		–		–							
N. flavescens NRL 30031 [MW166319]	–		–		–							
N. perflava NRL 9292 [MW166320]	–		–		–							
N. subflava ATCC 49275	–		–		–							
N. subflava ATCC 14221	–		–		–							
N. subflava NRL 9992 [MW166321]	–		–		–							
Other isolates												
N. bacilliformis ATCC BAA-1200	–		–		–							
N. elongata ATCC 25295	–		–		–							
N. polysaccharea ATCC 43768	–		–		–							
N. animalis ATCC 49930 (guinea pig)	–		–		–							
N. canis ATCC 14687 (dog)	–		–		–							
N. denitrificans ATCC 14686 (guinea pig)	–		–		–							
N. weaveri ATCC 51223 (dog bite)	–		–		–							

[1]*All species were isolated from human hosts unless otherwise indicated in parentheses.*
[2]*Species groups are as proposed by Bennett et al. (2012).*
[3]*Genbank accession numbers for rplF sequences generated in this study are given in brackets.*
[4]*N. mucosa NRL 9300 also displayed activity against each of these strains in the cross streak assay.*
CS, cross streak assay; +, zone of inhibition present; –, zone of inhibition absent; no symbol, not done.
AO, agar overlay assay; values indicate average diameter of zone of inhibition (mm); –, zone of inhibition absent; no symbol, not done.
n = 3 for each assay against each target.

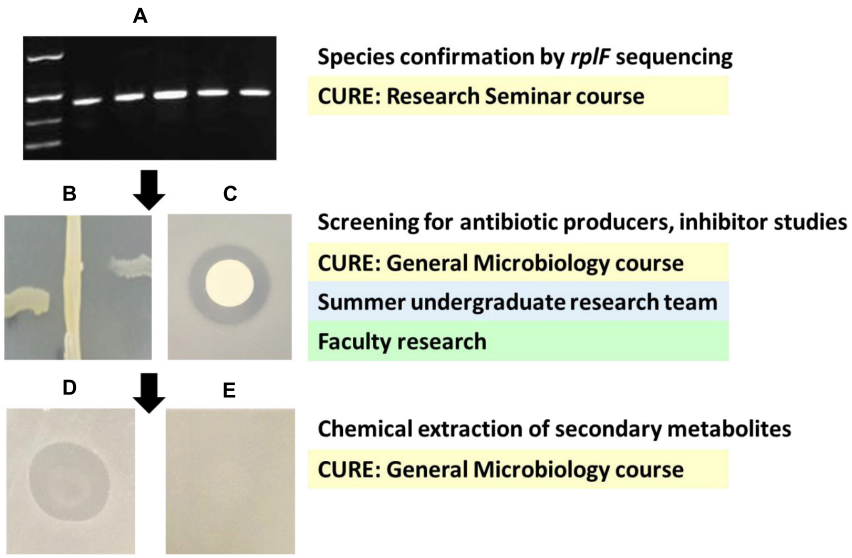

FIGURE 1 | Project design and example data from each project phase. Undergraduate involvement in each phase is given in shaded boxes. **(A)** Products of *rplF* amplification. Left to right: size standard (bottom to top: 300, 400, 500, and 750 bp), PCR products from *N. mucosa* NRL 9300, *N. mucosa* NRL 9297, *N. mucosa* ATCC 25996, *N. sicca* NRL 30016, and *N. sicca* ATCC 29256. **(B)** Cross streak assay of *N. mucosa* NRL 9300 inhibiting *N. flavescens* N47 (left of center) and *N. gonorrhoeae* ATCC 43070 (right of center), $n = 3$. **(C)** Agar overlay assay of a colony of *N. mucosa* NRL 9300 inhibiting the growth of a lawn of *N. flavescens* N47, $n = 3$. **(D)** Agar overlay assay of a purified ethyl acetate extract prepared from *N. mucosa* NRL 9300 inhibiting the growth of a lawn of *N. gonorrhoeae* FA6140, $n = 5$. **(E)** Control agar overlay assay of an ethyl acetate extract prepared from uninoculated GCB agar tested against a lawn of *N. gonorrhoeae* FA6140, $n = 5$.

onto GCB agar. After 24 h of incubation, 10 ml of melted GCB agar containing 10^6 CFU of a target strain was poured onto each plate and plates were reincubated. The diameter of the zone of inhibition surrounding the original producer strain was measured at 24 h (n = at least three trials per strain for each target). The number of CFU in the producer and target strain suspensions was confirmed by standard serial dilution and culture techniques.

Inhibitor Studies and Chemical Extraction

Inhibitor studies were used to investigate the antimicrobial activity displayed by *N. mucos*a NRL 9300 against *N. gonorrhoeae* strains MS11 and FA6140. Agar overlay assays in which the overlay contained 5, 50, or 500 U bovine catalase (Worthington Biochemicals) per ml or 500 μg bovine pancreatic trypsin (Sigma-Aldrich) per ml were performed to inhibit H_2O_2 (St. Amant et al., 2002) and protein (Jyssum and Allunans, 1982), respectively. Assays in which base and top agar contained 30 mM HEPES buffer (Sigma Life Sciences) pH 7.4 were used to inhibit activity due to acidification (Knapp et al., 1975). All inhibitor assays were performed in triplicate and zones of inhibition were compared to control plates lacking inhibitors. We also tested for the possibility of DNA mediated antimicrobial activity in our agar overlay assay by testing *N. mucosa* NRL 9300 against a Δ*comP* mutant strain of *N. gonorrhoeae* MS11 that is incapable of DNA uptake. Controls for this assay included wild type strain *N. gonorrhoeae* MS11 and a complemented Δ*comP* mutant strain. All MS11 strains were generously provided by Dr. Magdalene So (Kim et al., 2019).

A crude extract containing the *N. mucosa* NRL 9300 antimicrobial compound was isolated using the technique to enrich for small, nonpolar secondary metabolites described by Hernandez et al. (2018). Briefly, a lawn of plate grown bacteria and their secreted substances were harvested by chopping the agar from a 100 mm diameter petri plate into approximately 1 cm^2 pieces, freezing the material at −70°C, and performing an ethyl acetate extraction. The organic layer was dried, resuspended in methanol, and 30 μl samples containing 300 μg of extract, which represented approximately 20% of the yield, were spotted on an agar plate, allowed to dry, and tested against *N. gonorrhoeae* FA6140 using an agar overlay technique (n = 5). Control extractions were performed using uninoculated GBC agar (all chemicals were from Fisher Scientific).

RESULTS

N. mucosa Isolates Secrete Antimicrobial Compounds

We examined a panel of 36 nonpathogenic *Neisseria* isolates representing 15 species. The collection included 32 strains isolated from the human oropharynx, including reference strains utilized in the Human Microbiome Project (HMP) (Human Microbiome Jumpstart Reference Strains Consortium, 2010), and four strains from nonhuman animals. Although all strains were obtained from established isolate collections, the NRL strains are less well studied and their species identities had not been previously confirmed by genetic analyses. We used a validated technique to analyze a 413-bp region of the neisserial *rplF* gene,

which encodes the 50S ribosomal protein L6 (Bennett et al., 2014), from these strains and assigned isolates to neisserial species groups proposed by Bennett et al. (2012) (**Table 1**).

Delayed antagonism assays were used to test *Neisseria* species for their ability to produce antimicrobial compounds (**Table 1**, representative experiments shown in **Figure 1**). A semiquantitative cross-streak assay was used to screen all 36 isolates against three target strains: *N. lactamica* ATCC 23970, *N. flavescens* N47, and *N. gonorrhoeae* ATCC 43070. Five strains, all of which are members of the *N. mucosa* species group, displayed antimicrobial activity, although they did not exhibit identical activity profiles with respect to the target strains they inhibited (**Table 1**). Members of other neisserial species groups failed to show antineisserial activity under these conditions. Confirmatory agar overlay assays against the same three targets were carried out on all members of the *N. mucosa group*, yielding activity profiles identical to those seen in the initial screening experiments. Agar overlay zone of inhibition measurements suggest the *N. mucosa* producer strains may also differ from one another in their levels of antimicrobial activity, although these data should be interpreted with caution due to possible strain specific differences in growth rate and the limited quantitative power of this assay. The producer strain active against all screening targets, *N. mucosa* NRL 9300, was further tested against six additional *N. gonorrhoeae* strains, including the antibiotic resistant strain FA6140 (Veal et al., 2002), and displayed comparable activity against all gonococci in both assays (**Table 1**). Collectively, these data show nonpathogenic *Neisseria* species vary in their ability to secrete inhibitory compounds, and some *N. mucosa* strains are active against *N. gonorrhoeae*.

N. mucosa NRL 9300 Activity Is Not Affected by Common Inhibitors and Can Be Isolated by Chemical Extraction

Inhibitor studies were used to characterize the antimicrobial activity displayed by *N. mucosa* NRL 9300. Agar overlay assays against the target strains *N. gonorrhoeae* MS11 and FA6140 were performed in the presence of catalase, trypsin, and HEPES buffer. *N. mucosa* NRL 9300 displayed activity identical to that seen on control plates lacking inhibitor in all cases (**Supplementary Table 1**), suggesting the inhibitory activity is not due to H_2O_2, a trypsin sensitive protein toxin, or acidification. Kim et al. (2019) showed nonpathogenic *Neisseria* can kill *N. gonorrhoeae* in coculture assays via a mechanism in which *N. gonorrhoeae* takes up commensal DNA. These authors also demonstrated gonococcal DNA uptake mutants deficient in the protein ComP are no longer killed by this mechanism. We tested *N. mucosa* NRL 9300 inhibitory activity against a Δ*comP* mutant strain of *N. gonorrhoeae* MS11 using our agar overlay assay. *N. mucosa* inhibited the mutant strain to the same degree that it inhibited wild type MS11 and a complemented Δ*comP* mutant strain (**Supplementary Table 1**), indicating the inhibition detected by our delayed antagonism assays is not due to a DNA uptake mediated mechanism. Finally, an ethyl acetate extraction procedure was performed on plate-grown *N. mucosa* NRL 9300. The resulting crude extract inhibits *N. gonorrhoeae* strain FA6140 in an agar overlay assay (**Figure 1**), suggesting the antimicrobial activity displayed by *N. mucosa* may be due to a novel secondary metabolite.

UNDERGRADUATE INVOLVEMENT AND TRANSFERRABLE STRATEGIES

This project was designed to provide engaging undergraduate research experiences to as many students as possible while advancing the scientific objectives of the study. Although assessment of student learning outcomes was not a component of this work, a brief description of the project design and phasing from a pedagogical perspective may serve as a helpful model to others interested in designing human microbiome based antibiotic discovery experiences in undergraduate settings (**Figure 1**). The work described above involved over 70 students enrolled in three undergraduate biology classes in course-based undergraduate research experiences (CUREs) and provided a full-time summer research opportunity to a team of two students. Straightforward, relatively low-cost techniques were used throughout the project. Students involved in CUREs worked exclusively with nonpathogenic bacteria that could be handled safely in the teaching laboratory environment, while the faculty member and summer team carried out experiments utilizing *N. gonorrhoeae* under more stringent training and safety conditions. Participants in each research phase learned about the overarching goals of the neisserial antibiotic discovery project, and studied data generated by previous teams and relevant primary literature. Students in an upper-level genomics focused Research Seminar course carried out the DNA sequence based strain characterization in conjunction with learning about PubMLST and other databases, which they subsequently used to carry out additional independent bioinformatics projects. Participants in a General Microbiology course composed largely of students interested in health professions carried out initial screening of the isolate collection for inhibitory activity against the nonpathogenic target strains. This course represents students' first exposure to working with bacteria in the laboratory. Participation in this study provided students with opportunities to learn not only the assays used in the project, but also basic microbiology techniques such as media preparation, streak plating, and maintaining pure cultures, in the context of an engaging research question. In addition, the project complemented classroom activities addressing symbiosis, the human microbiome, and antibiotic resistance. The summer research team, which was composed of a first year student, a third year student, and the faculty member, replicated these assays, developed the agar overlay confirmatory assay, included screening against *N. gonorrhoeae*, and carried out inhibitor studies. Finally, *N. mucosa* chemical extractions were incorporated into a subsequent section of General Microbiology. Students presented their findings from various stages of the project to the broader campus community as part of a yearly Celebration of Student Scholarship, and the summer team also presented at regional and national conferences. This phased

model blending faculty research interests, course goals, and both introductory and upper-level of undergraduate research involvement has allowed a broad range of students with various levels of experience to engage with and contribute to this project.

Our laboratory has a long history of *Neisseria* research, but this undergraduate antibiotic discovery model could easily be adapted to the study of other members of the human microbiome. The NIH Human Microbiome Project included both metagenomic analyses of body site samples from human volunteers and sequencing of approximately 800 previously isolated reference strains representing an array of species found in the microbiome (Human Microbiome Jumpstart Reference Strains Consortium, 2010; Human Microbiome Project Consortium, 2012). The HMP reference strains are well described in a searchable catalog[2] and many are readily available from commercial strain collections. While the choice to screen HMP reference strains and/or other known strain collections for antibiotic production limits the number of representatives of the microbiome one can test, it has the advantage of avoiding the need for human subject research approval and safeguards that might be challenging in some undergraduate settings. Additionally, the ready availability of many reference strain genome sequences opens the possibility of linking functional screening to bioinformatics projects related to antibiotic discovery.

DISCUSSION

Bacteria residing in a common habitat display a wide array of competitive and cooperative interactions. The human oropharynx is home to over 700 prokaryotic species, including at least 12 *Neisseria* species, which are in intimate contact with one another, and with their host, in variety of niches (Liu et al., 2015). Several recent studies utilizing metagenomic data from the HMP have demonstrated *N. mucosa* is a common colonizer of the healthy oropharynx and displays niche specificity for sites including the buccal mucosa and supragingival plaque (Eren et al., 2014; Kraal et al., 2014; Mark Welch et al., 2014, 2016; Donati et al., 2016). For example, Kraal et al. (2014) reported supragingival plaque contained *N. mucosa* in 70/70 subjects tested, although the abundance of the organism varied among subjects. The normal mutualistic roles *N. mucosa* and other *Neisseria* species play as members of the healthy human microbiome are not well understood, however, we reasoned that nonpathogenic *species* may secrete secondary metabolites active against other *Neisseria*. Even though our study focused on a relatively small panel of isolates, we identified five *N. mucosa* group strains exhibiting antimicrobial activity against tester strains of nonpathogenic *Neisseria*, three of which also displayed activity against *N. gonorrhoeae*. This activity was retained in a crude ethyl acetate extract enriched for small nonpolar secondary metabolites.

The *N. mucosa* antigonococcal activity we observed does not appear to be due to previously described inhibitory mechanisms. Other genera residing in human microbiome, including vaginal *Lactobacillus* species, also display activity against *N. gonorrhoeae in vitro*. *Lactobacillus*-mediated inhibition is mediated by H_2O_2, bacteriocins, organic acids, or a combination of the three (St. Amant et al., 2002; Graver and Wade, 2011; Ruíz et al., 2015; Foschi et al., 2017). Inhibitors of these agents had no effect on *N. mucosa* antigonococcal activity. We also used DNA uptake mutants of *N. gonorrhoeae* to rule out the possibility we were detecting DNA mediated gonococcal killing previously described in nonpathogenic *Neisseria* (Kim et al., 2019). These findings suggest further characterization of the compounds secreted by *N. mucosa* NRL 9300 is warranted and raise the possibility nonpathogenic *Neisseria* may employ multiple mechanisms for inhibiting other bacteria. This would not be surprising given that each unique nonpathogenic species interacts with a variety of other organisms in a diverse array of dynamic body site habitats.

Natural products have not been well studied in the genus *Neisseria* as a whole, and to the best of our knowledge this is the first report describing secondary metabolite activity in *N. mucosa*. Future directions for this project include chemical and quantitative analyses of the components of the *N. mucosa* ethyl acetate extract, validation of antigonoccal activity in animal models, and examination of neisserial genomes for potential biosynthetic gene clusters. The antibiotic discovery potential we have identified in *Neisseria* species is likely also present in many other uncharacterized members of the human microbiome that await investigation. This demonstration project has shown undergraduate teams participating in CUREs and other guided research experiences can make continued contributions to this emerging area of natural products research.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

EA, JO, and AF designed and conducted experiments and interpreted the data. EA developed the overall project, directed course-based research, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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[2]https://www.hmpdacc.org/hmp/catalog/

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.577762/full#supplementary-material

REFERENCES

Bennett, J. S., Jolley, K. A., Earle, S. G., Corton, C., Bentley, S. D., Parkhill, J., et al. (2012). A genomic approach to bacterial taxonomy: an examination and proposed reclassification of species within the genus *Neisseria*. *Microbiology* 158, 1570–1580. doi: 10.1099/mic.0.056077-0

Bennett, J. S., Watkins, E. R., Jolley, K. A., Harrison, O. B., and Maiden, M. C. (2014). Identifying *Neisseria* species by use of the 50S ribosomal protein L6 (rplF) gene. *J. Clin. Microbiol.* 52, 1375–1381. doi: 10.1128/JCM.03529-13

Centers for Disease Control and Prevention (2012). *Cephalosporin-Resistant Neisseria Gonorrhoeae Public Health Response Plan*. Atlanta: CDC.

Custodio, R., Johnson, E., Liu, G., Tang, C. M., and Exley, R. M. (2020). Commensal *Neisseria cinerea* impairs *Neisseria meningitidis* microcolony development and reduces pathogen colonisation of epithelial cells. *PLoS Pathog.* 16:e1008372. doi: 10.1371/journal.ppat.1008372

Deasy, A. M., Guccione, E., Dale, A. P., Andrews, N., Evans, C. M., Bennett, J. S., et al. (2015). Nasal inoculation of the commensal *Neisseria lactamica* inhibits carriage of *Neisseria meningitidis* by young adults: a controlled human infection study. *Clin. Infect. Dis.* 60, 1512–1520. doi: 10.1093/cid/civ098

Donati, C., Zolfo, M., Albanese, D., Tin Truong, D., Asnicar, F., Iebba, V., et al. (2016). Uncovering oral *Neisseria* tropism and persistence using metagenomic sequencing. *Nat. Microbiol.* 1:16070. doi: 10.1038/nmicrobiol.2016.70

Donia, M. S., and Fischbach, M. A. (2015). Small molecules from the human microbiota. *Science* 349, 1254766. doi: 10.1126/science.1254766

Dube, D. H. (2018). Design of a drug discovery course for non-science majors. *Biochem. Mol. Biol. Educ.* 46, 327–335. doi: 10.1002/bmb.21121

Eren, A. M., Borisy, G. G., Huse, S. M., and Mark Welch, J. L. (2014). Oligotyping analysis of the human oral microbiome. *Proc. Natl. Acad. Sci. U.S.A.* 111, E2875–E2884. doi: 10.1073/pnas.1409644111

European Centre for Disease Prevention and Control (2019). *Response Plan to Control and Manage The Threat of Multi- and Extensively Drug-Resistant Gonorrhoeae in Europe – 2019 Update*. Stockholm: ECDC.

Eyre, D. W., Sanderson, N. D., Lord, E., Regisford-Reimmer, N., Chau, K., Barker, L., et al. (2018). Gonorrhea treatment failure caused by a *Neisseria gonorrhoeae* strain with combined ceftriaxone and high-level azithromycin resistance, England, February 2018. *Euro Surveill.* 23:1800323. doi: 10.2807/1560-7917.ES.2018.23.27.1800323

Foschi, C., Salvo, M., Cevenini, R., Parolin, C., Vitali, B., and Marangoni, A. (2017). Vaginal lactobacilli reduce *Neisseria gonorrhoeae* viability through multiple strategies: an *in vitro* study. *Front. Cell. Infect. Microbiol.* 7:502. doi: 10.3389/fcimb.2017.00502

Genné-Bacon, E. A., and Bascom-Slack, C. A. (2018). The PARE Project: a short course-based research project for national surveillance of antibiotic-resistant microbes in environmental samples. *J. Microbiol. Biol. Educ.* 19, 19–97. doi: 10.1128/jmbe.v19i3.1603

Graver, M. A., and Wade, J. J. (2011). The role of acidification in the inhibition of *Neisseria gonorrhoeae* by vaginal lactobacilli during anaerobic growth. *Ann. Clin. Microbiol. Antimicrob.* 10:8. doi: 10.1186/1476-0711-10-8

Hernandez, S., Tsang, T., Bascom-Slack, C., Broderick, N., and Handelsman, J. (2018). *Tiny Earth: A Research Guide to Studentsourcing Antibiotic Discovery*. Acton, MA: XanEdu Press.

Hoskisson, P. A., Aldridge, P., and Bowater, L. (2015). Inspiring STEM undergraduates to tackle the AMR crisis. *FEMS Microbiol. Lett.* 362:fnv138. doi: 10.1093/femsle/fnv138

Human Microbiome Jumpstart Reference Strains Consortium, Nelson, K. E., Weinstock, G. M., Highlander, S. K., Worley, K. C., Creasy, H. H., et al. (2010). A catalog of reference genomes from the human microbiome. *Science* 328, 994–999. doi: 10.1126/science.1183605

Human Microbiome Project Consortium (2012). A framework for human microbiome research. *Nature* 486, 215–221. doi: 10.1038/nature11209

Jolley, K. A., Bray, J. E., and Maiden, M. (2018). Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res.* 3:124. doi: 10.12688/wellcomeopenres.14826.1

Jyssum, K., and Allunans, J. (1982). Three types of growth inhibition among strains of *Neisseria meningitidis* isolated from patients in Norway. *Acta Pathol. Microbiol. Immunol. Scand. B* 90, 335–340. doi: 10.1111/j.1699-0463.1982.tb00128.x

Kellogg, D. S., Peacock, W. L., Deacon, W. E., Brown, L., and Pirkle, D. I. (1963). *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. *J. Bacteriol.* 85, 1274–1279.

Kim, W. J., Higashi, D., Goytia, M., Rendón, M. A., Pilligua-Lucas, M., Bronnimann, M., et al. (2019). Commensal *Neisseria* kill *Neisseria gonorrhoeae* through a DNA-dependent mechanism. *Cell Host Microbe* 26, 228–239. doi: 10.1016/j.chom.2019.07.003

Knapp, J. S., Falkow, S., and Holmes, K. K. (1975). Reevaluation of bacteriocinogeny in *Neisseria gonorrhoeae*. *J. Clin. Pathol.* 28, 274–278. doi: 10.1136/jcp.28.4.274

Kraal, L., Abubuker, S., Kota, K., Fischbach, M. A., and Mitreva, M. (2014). The prevalence of species and strains in the human microbiome: a resource for experimental efforts. *PLoS One* 9:e97279. doi: 10.1371/journal.pone.0097279

Liu, G., Tang, C. M., and Exley, R. M. (2015). Non-pathogenic *Neisseria*: members of an abundant, multi-habitat, diverse genus. *Microbiology* 161, 1297–1312. doi: 10.1099/mic.0.000086

Maiden, M. C. (1998). Horizontal genetic exchange, evolution, and spread of antibiotic resistance in bacteria. *Clin. Infect. Dis.* 27(Suppl. 1), S12–S20. doi: 10.1086/514917

Mark Welch, J. L., Rossetti, B. J., Rieken, C. W., Dewhirst, F. E., and Borisy, G. G. (2016). Biogeography of a human oral microbiome at the micron scale. *Proc. Natl. Acad. Sci. U.S.A.* 113, E791–E800. doi: 10.1073/pnas.1522149113

Mark Welch, J. L., Utter, D. R., Rossetti, B. J., Mark Welch, D. B., Eren, A. M., and Borisy, G. G. (2014). Dynamics of tongue microbial communities with single-nucleotide resolution using oligotyping. *Front. Microbiol.* 5:568. doi: 10.3389/fmicb.2014.00568

Marri, P. R., Paniscus, M., Weyand, N. J., Rendón, M. A., Calton, C. M., Hernández, D. R., et al. (2010). Genome sequencing reveals widespread virulence gene exchange among human *Neisseria* species. *PLoS One* 5:e11835. doi: 10.1371/journal.pone.0011835

Milshteyn, A., Colosimo, D. A., and Brady, S. F. (2018). Accessing bioactive natural products from the human microbiome. *Cell Host Microbe* 23, 725–736. doi: 10.1016/j.chom.2018.05.013

Rowley, J., Vander Hoorn, S., Korenromp, E., Low, N., Unemo, M., Abu-Raddad, L. J., et al. (2019). Chlamydia, gonorrhea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. *Bull. World Health Organ.* 97, 548–562. doi: 10.2471/BLT.18.228486

Ruíz, F. O., Pascual, L., Giordano, W., and Barberis, L. (2015). Bacteriocins and other bioactive substances of probiotic lactobacilli as biological weapons against *Neisseria gonorrhoeae*. *Pathog. Dis.* 73:ftv013. doi: 10.1093/femspd/ftv013

Smyth, D. S. (2017). An authentic course-based research experience in antibiotic resistance and microbial genomics. *Sci. Educ. Civic Engag.* 9, 59–65.

Spratt, B. G., Bowler, L. D., Zhang, Q. Y., Zhou, J., and Smith, J. M. (1992). Role of interspecies transfer of chromosomal genes in the evolution of penicillin resistance in pathogenic and commensal *Neisseria* species. *J. Mol. Evol.* 34, 115–125. doi: 10.1007/BF00182388

St. Amant, D. C., Valentin-Bon, I. E., and Jerse, A. E. (2002). Inhibition of *Neisseria gonorrhoeae* by *Lactobacillus* species that are commonly isolated from the female genital tract. *Infect. Immun.* 70, 7169–7171. doi: 10.1128/iai.70.12.7169-7171.2002

Unemo, M., Seifert, H. S., Hook, E. W. III, Hawkes, S., Ndowa, F., and Dillon, J. R. (2019). Gonorrhoea. *Nat. Rev. Dis. Primers* 5:79. doi: 10.1038/s41572-019-0128-6

Veal, W. L., Nicholas, R. A., and Shafer, W. M. (2002). Overexpression of the MtrC-MtrD-MtrE efflux pump due to an *mtrR* mutation is required for chromosomally mediated penicillin resistance in *Neisseria gonorrhoeae*. *J. Bacteriol.* 184, 5619–5624. doi: 10.1128/jb.184.20.5619-5624.2002

Weyand, N. J. (2017). *Neisseria* models of infection and persistence in the upper respiratory tract. *Pathog. Dis.* 75:ftx031. doi: 10.1093/femspd/ftx031

Whiley, D. M., Jennison, A., Pearson, J., and Lahra, M. M. (2018). Genetic characterisation of *Neisseria gonorrhoeae* resistant to both ceftriaxone and azithromycin. *Lancet Infect. Dis.* 18, 717–718. doi: 10.1016/S1473-3099(18)30340-2

Wilson, M. R., Zha, L., and Balskus, E. P. (2017). Natural product discovery from the human microbiome. *J. Biol. Chem.* 292, 8546–8552. doi: 10.1074/jbc.R116.762906

World Health Organization, Department of Reproductive Health and Research (2012). *Global Action Plan to Control the Spread and Impact of Antimicrobial Resistance in Neisseria gonorrhoeae*. Geneva: WHO.

Zipperer, A., Konnerth, M. C., Laux, C., Berscheid, A., Janek, D., Weidenmaier, C., et al. (2016). Human commensals producing a novel antibiotic impair pathogen colonization. *Nature* 535, 511–516. doi: 10.1038/nature18634

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PERSPECTIVE
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Bringing Real-World Microbiology Experiences to Undergraduate Students in Resource-Limited Environments

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Undergraduate microbiology curriculum should be amenable to periodic changes to incorporate new developments and ideas. The curriculum should be used not merely as a way to disseminate facts but also as a way to allow students to experience the process of science. In the context of undergraduate microbiology education in Osmania University (Hyderabad, India), existing curriculum does not explicitly allow students to engage in deeper understanding of concepts and understanding of the process of science, both in lecture and laboratory courses. The assessment methods that are currently used are limited in scope as they only test factual recall and superficial understanding of the subject and very minimally assess critical thinking skills. Another factor hampering innovation in the broader context of undergraduate education is the unavailability and inaccessibility to adequate resources. To address the issue of resource-limitations in implementing activities that expose undergraduate students to real-world microbiology experiences, a collaboration between a research institute and two teaching colleges was formed. This collaboration involved teacher and student workshops on exploring microbial diversity using 16S rRNA analysis with a view of blending novel research questions with technical skills in the undergraduate microbiology lab. This effort is an example of educators providing students with authentic experiences and, helping them gain critical knowledge and research skills in microbiology even under resource constraints, and students demonstrating motivation to participate in similar activities in the future. The collaborative effort described here can be a broadly sustainable model to improve overall undergraduate education in relatively resource-limited environments.

Keywords: microbial diversity, undergraduate academic success, resource limitations, faculty professional development, student workshops, primary research articles

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INTRODUCTION

The goals of undergraduate biology education can range from enabling students to gain broad knowledge and experiences that will prepare them to be become socially conscious citizens who can effectively contribute to the needs of the community (Ferren and Anderson, 2016; Hatcher, 2011; Association of American Colleges and Universities, 2007; Penn, 2011; Zai, 2015). Learning

spaces should be engaging, enriching and empowering in order to help students achieve these goals. Real world experiences are inherently rich in context, have the power to engage and are relevant. Bringing these real-world experiences into classrooms can significantly improve the quality of teaching and learning (Alberts, 2005).

Basic science education in India at the undergraduate level, unlike technical and professional education, predominantly happens not on University campuses but in over 10,000 colleges affiliated to degree granting Universities under the purview of the University Grants Commission (Government of India and University Grants Commission [UGC], 2019). With a greater emphasis on didactic classroom instruction, and constrained by resource limitations, it is believed that most of these colleges are unable to provide adequate training to their students to meet the needs of academia and industry. In addition, existing problems with the current higher education system in India pointed by Saberwal (2019), pose a challenge to the introduction of newer ideas and methods, especially in biology education. In a meeting report on "Policy framework for catalyzing excellence in science education and research in India" (Lakhotia et al., 2013), the authors state that "...a rejuvenation of the existing undergraduate and postgraduate science education system together with an integration of teaching with high-quality research is also desperately needed" and point to a steady decline of the infrastructure, quality of faculty and the research capacity of the higher educational system as a whole for over three decades. One of their specific recommendations for improving the current state of undergraduate education is to recognize good teaching practices and development of open-ended laboratory work/research projects by students.

While a top down approach via policy statements and national plans have their role in transforming undergraduate education, true transformation is only possible when individual colleges and informed teachers are empowered to take action. Under the purview of University Grants Commission of India (UGC), which is charged with coordination, determination and maintenance of standards of higher education in India, out of all educational institutions in India, about 750 colleges are autonomous allowing them to design and implement small courses or programs by themselves and supplement the University prescribed syllabus. This autonomy allows administrators and teachers the freedom to experiment and pursue alternative pedagogies to develop innovative and exemplary teaching practices and create rich open-ended learning experiences for students.

While extended research internships in an apprentice model are highly desired to provide rich and comprehensive experiences to students, they are not always possible and scalable due to resource constraints. Studies show that well designed shorter experiences can have equally enriching experiences for students (Wei and Woodin, 2011; Frantz et al., 2017). Here, we describe a program developed through a collaboration between a research institute and teachers at two private autonomous colleges to expose undergraduate students majoring in microbiology to real-world microbiology experiences in Hyderabad, India.

PROFESSIONAL DEVELOPMENT PROGRAM – CATALYST FOR TRANSFORMATION

A series of teacher (faculty development) workshops developed by the Center for Advancement of Research Skills (CARS) at Dr. Reddy's Institute of Life Sciences (Hyderabad, India) initiated a dialog between its researchers and undergraduate educators in the local colleges to create specific interventions that would result in changes with respect to student engagement and learning outcomes in college classrooms. Typically, faculty professional development workshops are focused either on discipline-specific content or exclusively on pedagogy. The CARS workshop series made a deliberate attempt to blende these two aspects and placed emphasis on teachers sharing their skill and experiences with their students. The workshops, which were conducted at the research institute, involved teachers assuming student personas to experience a research project while discussing ideas in pedagogy and potential challenges in classroom implementation. The final aspect of the workshops was to create classroom modules or co-curricular activities that they could implement in their respective institutions. A 3-day hands-on workshop on "Exploring Microbial Diversity" was organized for educators with an explicit goal of enabling them to plan and implement student programs that align closely to the existing microbiology curriculum in their respective colleges. Subsequent to the workshop, the research institute provided continuous support to the teachers, as needed, throughout the duration of the student programs in the colleges.

CLASSROOM IMPLEMENTATION

The current undergraduate degree programs in the sciences are offered as 3-year programs in India. Students opt for specific combinations of subjects upon enrolling into college and continue with the same combination for 3 years. For example, students interested in microbiology can select combinations such as Microbiology, Botany, Chemistry (MBC) or Microbiology, Biochemistry and Chemistry (MBiC), Microbiology, Genetics, Chemistry (MGC), or Microbiology, Zoology, Chemistry (MZC). The syllabus for the courses taught in the above programs is framed and prescribed by the Boards of Studies at the degree-granting University. While University constituents and affiliated colleges are mandated to strictly follow these syllabi, the autonomous colleges (which are a focus of this study) have some flexibility in modifying their curricula and syllabi. This also includes the control of 20–30% of student grades within the college, through internal assessments. This autonomy allowed us the freedom to – design and implement the "Exploring Microbial Diversity" workshop for undergraduate students in two colleges.

Following the teacher workshop, we designed a laboratory workflow that is likely to succeed during classroom implementation, keeping in view the academic semester schedules, alignment with the syllabus and available material resources in the colleges. There were differences between the two colleges that resulted

TABLE 1 | Outline of the collaborative program development in two phases.

	Phase I	Phase 2		
			College 1	College 2
		Microbiology majors	100 × 3 years = 300	90 × 3 years = 270
		Women	154 (51%)	270 (100%)
		Men	146 (48.6%)	0 (0%)
	Exploring Microbial Diversity by 16S rRNA profiling			
Activity	Teacher workshop		Student workshop	Student workshop
Organizer	Research Scientist		College Teachers (5)	Research Scientist + Teaching Assistant
Venue	Research Institute		Bhavan's Vivekananda College	St. Ann's College for Women
Participants	College Teachers (4)		BSc II year students (30) 23 women; 7 men	BSc I and III year students (15) 13 I year; 2 III year
			6 students per team	3 students per team
Duration	3 days (6 h per day)		1 week (4 h per day)*	2 weeks (2 h per day)**
Assessment	Implementation of student workshops in the colleges		Informal conversations, self-reported student feedback	Poster presentation at the research institute, self-reported student feedback

*Phase I included an undergraduate teacher workshop followed by Phase II where student workshops were conducted at two colleges. * The workshop was split into two sessions of 2 h, in the morning prior to regular classes and in the afternoon after formal college hours; **the workshop was conducted at the end of the formal college hours.*

in unique challenges for classroom implementation. Apart from structural constraints, there were inclusivity issues with respect to student enrollments into the workshop. Since the activities would be conducted beyond the official college hours, only those students who could and chose to stay beyond regular class time could enroll in the workshop (**Table 1**). With regards to the instructional team, two undergraduate instructors from one college who participated in the teacher workshop trained three additional instructors at their own institution to strengthen the program with adequate resources by all means. All the five instructors who were conversant with the laboratory workflow conducted a 3-day trial run at the college to test and ensure proper functioning of chemicals, reagents and equipment. Critical reagents and consumables were supplied by the research institute while the colleges provided the basic laboratory infrastructure. With respect to instruments, micropipette sets, a miniPCR^{TM} thermocycler, a blueGel^{TM} gel electrophoresis unit and a PCR microcentrifuge were made available on loan from the research institute. They were extensively used in one college while the other college did not need all of them to run the workshops.

EXPLORING MICROBIAL DIVERSITY IN THE LABORATORY

One of the specific goals of this program was to integrate practical laboratory experiments prescribed in the University syllabus into a research project, changing the focus from laboratory techniques to investigating authentic, real-world biological questions (**Supplementary Table 1**). This program was designed as a short and intense 1- to 2-week workshop focused on exploring bacterial diversity using 16S RNA T-RFLP workflow (Schütte et al., 2008). Similar workflows can be seen in successful programs such as Small World Initiative and Tiny Earth (Basalla et al., 2020), and Urban Barcode Project (Henter et al., 2016). Students at both colleges worked in teams to explore bacterial diversity in an environmental sample of their choice. They were required to provide reasons for their choice of samples brought for analysis; the samples included raw unpasteurized milk, spoiled fruits and vegetables, cockroach gut, water and soil from a lake and a polluted local river (Musi), iron rust, compost pit, cow dung and effluent from a local chemical industry. All microbial cultures were handled under aseptic conditions and proper lab safety measures were ensured starting from inoculation of cultures to their eventual disposal by autoclaving; safety considerations were discussed before each step of the workflow was executed. After practicing the micropipetting technique and making serial dilutions, students streaked the samples on LB media plates to isolate single bacterial colonies and use those single colonies to perform PCR amplification of the 16S rRNA gene locus using Universal primers (27F and 1492R; Frank et al., 2008). PCR amplicons thus obtained were analyzed using either agarose or polyacrylamide gel electrophoresis (PAGE) before and after digestion with restriction endonucleases such as AluI, DpnI, MboI, and TaqI, which are frequent (4 bp) cutters. The banding patterns corresponding to different bacterial colonies were compared to infer diversity. A brief introduction to genome databases and bioinformatics tools enabling sequence analysis was also given toward the end of the program. *In silico* restriction digestion of 16S rRNA gene sequences from different bacterial species using NEB/web cutter[1] allowed the students to compare their experimental results with computational analysis. This workflow allowed us to cover several molecular methods and techniques that undergraduate microbiology students benefit from learning and are part of the prescribed syllabus.

Despite the short duration, students were not only able to go through the complete workflow but were able to collect

[1]http://nc2.neb.com/NEBcutter2/

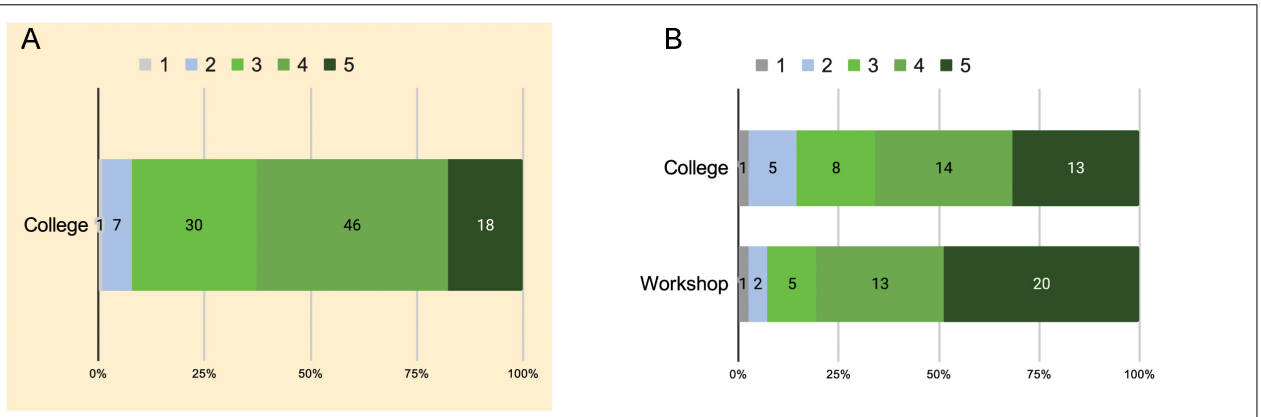

FIGURE 1 | Stacked bar graph showing student rating the impact of college courses and "Exploring Microbial Diversity" workshop on their research and scientific attitudes and skills. **(A)** Rating of college experiences by students who did not participate in the workshop (control) and **(B)** rating of college and workshop experiences by student participants. 1 corresponds to the lowest rating and 5 to the highest rating.

data and document their findings. The collected data included geographical location and nature of samples, serial dilutions and the corresponding density of bacterial colonies on media plates, presence of PCR amplified 16S rRNA gene fragment after gel electrophoresis, observed RFLP patterns, and simulated RFLP patterns using gene sequences from genomic databases. Documentation of their findings became especially important since students had to present the summary of their work at the conclusion of the activity.

Alongside the content of the student activity, several pedagogical considerations were deliberated during the teacher workshop and implemented in the colleges. We made teamwork an essential component so as to enable peer interactions and enhance peer learning. Moreover, the entire activity was based on experiential, hands-on laboratory work beginning from sample collection, planning and executing individual steps of the experiments, and ending with comparing and discussing the results. Therefore, student interactions became essential for successful completion of the experiments and thus were ensured. During the course of the laboratory work, we emphasized on the process of science alongside the execution of instructions and protocols. Questions regarding the underlying principles and logical reasons behind various steps in the experimental protocol were raised at appropriate times.

IMPACT OF THE STUDENT WORKSHOPS ON STUDENT LEARNING

To understand and assess the impact of this program, we collected self-reported student feedback at the end of the workshop using a questionnaire with response options in the Likert-scale and open-ended questions. The questions aimed at understanding students' views on different aspects of the curriculum, teaching and learning practices, and gain of cognitive skills, both in their regular college courses and the workshop. Another set of first- and second-year students who did not attend the workshops were given a similar set of questions focusing only on their college experiences, and their anonymous responses were used as "controls." A total of 41 students from the two colleges who participated in the workshops (91% response rate) and 102 students from the "control" group completed the questionnaire.

With regards to rating the impact of college courses and the workshop on academic experiences, especially developing science identity and process skills, there is a clear distinction in the number of students who gave a rating of "5" (highest) attributed to regular courses/classes and the "Exploring Microbial Diversity" workshop; 49% of the workshop participants rated the workshop experience at "5"; 31.7% of the workshop participants rated the impact of their college experience at "5"; only 17.65% of the students in the control group rated the impact of college courses at "5" (**Figure 1**).

The questions focused on three broad categories of students' learning experiences; key questions from each category included (a) Emphasis, how much either the college or the workshop emphasized on memorization, analysis, synthesis and application (**Supplementary Figure 1**); (b) Impact, how either the college or the workshop helped them to think independently and analyze concepts (beyond memorization), actively engaged them with academic learning, enhancing their interest in the subject, and gave them full autonomy to plan and execute lab experiments (**Supplementary Figure 2**); and (c) Involvement, how well they prepared for regular college courses and for the workshop by reading study material, writing extensive notes, reading beyond the prescribed material, and discussing course material with classmates (**Supplementary Figure 3**). The analysis of all the responses generally indicated that the students gained more from the workshop experience, when compared to their experiences in regular classes. However, the responses do not allow us to draw specific conclusions with regards to differences between student "involvement" with various activities (**Supplementary Figure 3**).

When asked to comment (as free responses) on the differences they noticed between regular college classes and the workshop, students felt that the workshop enabled them to learn things which they were not able to do and analyze in

regular courses, made them to ask questions, think independently, understand concepts better and to apply them effectively. Many students mentioned that they would look forward to more such workshops during their undergraduate studies. One student comment captures the essence of many of these responses – "*Without doubt the "Exploring Microbial Diversity" workshop was better than classes as it involved doing something we do not usually do in classes. Practical experience is the best teacher. But I think a little emphasis on research papers for reference should be included as part of the [laboratory] practicals, and if possible, in classes, as that will not only intensify the research component of the subject but also will give fundamental exposure to scientific literature.*" An analysis of the responses represented in the stacked bar graphs, in conjunction with the free responses, suggests that the workshop experience had a positive impact on most of the students with respect to their engagement with discipline-specific content and also science process skills.

AN OUTSIDE PERSPECTIVE ON STUDENT PERFORMANCE

Students from one of the colleges (**Table 1**) had an opportunity to present their work to scientists (principal investigators, postdoctoral researchers, graduate students and research associates) at the research institute. This was the only interaction they had with the students. Judging from the presentations, in spite of the variation in the quality, the scientists felt that the students were well prepared, interested, enthusiastic, and demonstrated an ability to understand and critically analyze primary research and experimental findings.

TEACHER PERSPECTIVES

The teachers were aware of the limitations and shortcomings related to both material and time resources. Nevertheless, they were encouraged by the fact that we were able to create and implement these pilot programs to engage students, give them an opportunity to explore research questions and enable them to gain deeper insights into real-world microbiology. The key learnings from the first implementation of the "Exploring Microbial Diversity" workshops are that material limitations can be overcome with creative program design in collaboration with the research institute, and time limitations can be overcome by scheduling the programs around regular college hours.

DISCUSSION

There are several well documented microbiology education programs aimed at providing authentic experiences to undergraduate students (Jordan et al., 2014; Henter et al., 2016; Staub et al., 2016; Basalla et al., 2020). However, in India there are strong perceptions about the difficulty of implementing such programs to systematically improve undergraduate programs. These perceptions are strengthened in the light of poor infrastructure and inadequate facilities in colleges, and time constraints imposed by rigid curricular outcomes. Most of these challenges are likely to be common and widespread in similar academic settings, therefore we need to examine practical ways to overcome these challenges by creating focused college faculty development programs aimed at fostering collaborative programs with research institutes by way of sharing of their resources.

The pilot study described here provides a model for redesigning undergraduate microbiology education in resource-limited environments such as small, private, autonomous, University-affiliated colleges in India. The program focused on inquiry and problem solving in an environment that fosters teamwork and collaboration amongst students. In an academic system rife with intense competition, this is an alternative to expose students to working in teams and enhancing their learning experiences. It is vital that any of these efforts aimed at improving undergraduate STEM education should include rigorous designs and assessments that will help us evaluate learning outcomes of students and thereby the effectiveness of programs (Linn et al., 2015).

The success of high-standard abbreviated "nanocourses" that provide high-impact learning experiences to students (Bentley et al., 2008) provide templates upon which shorter, workshop-style courses can be developed and implemented that address the constraints of time and material resources. Scaffolding authentic research across the curriculum (Sieg et al., 2019) by way of short workshops can be an effective way to leverage academic autonomy and overcome the said constraints. With either internal support from college administration or external support through collaboration with research institutes, teachers can design workshops that cater to the knowledge and skills needs of students while spreading their efforts to implement these workshops. If limited physical presence in colleges is imposed, lecture/discussion-based instruction can happen using synchronous or asynchronous online platforms, while the in-person time can be spent in laboratories to gain important hands-on skills. Scientists in research laboratories are also likely to get involved and collaborate with college teachers to organize such short student programs.

The most significant factor that made this pilot study feasible is the autonomy (even in the limited sense) the participating colleges had in terms of creating and implementing small programs, which allowed the undergraduate instructors to engage in professional development at a research institute. The research institute's participation was rooted in their interest in ensuring that these students who are prospective recruits are well trained to fuel their future scientific research programs. The active collaboration between research institutes like Cold Spring Harbor Laboratory, Howard Hughes Medical Institute's (HHMI) Janelia Farms, and undergraduate teaching institutes have given rise to exemplary educational innovations. Similar partnerships, on a smaller scale, can be built and nurtured to support educational activities in local undergraduate institutions. In fact, these smaller partnerships are likely to be stronger and become successful because of greater connectedness. In India, research laboratories belonging to the Council for Scientific and Industrial Research

(CSIR), Department of Biotechnology (DBT), Indian Council for Medical Research (ICMR), Indian Council for Agricultural Research (ICAR) are spread across the country and have a wide variety of microbiology-related research programs, which can provide modest support through knowledge- and resource-sharing. This support can result in deep and far-reaching impact on the quality of undergraduate microbiology education.

As long as colleges are willing to find creative ways to use even a limited amount of autonomy at their disposal to innovate in their classrooms, and research institutions with greater resources at their disposal are willing to become enthusiastic supporters and enablers of high-quality educational experiences, there is immense opportunity for students to engage in authentic learning experiences.

CONCLUSION

With India unveiling a new National Education Policy in 2020, there is a promise to revamp the entire education landscape in the country. As alluded to earlier, while policies do have a top-down influence on the system, true change is possible when the teachers and colleges are developing and practicing exemplary work at the grassroots. The collaborative model showcased here has given the necessary impetus to the teachers to continue providing rich authentic, real-world microbiology experiences to undergraduate students. We hope that our modest effort and its positive impact on student experiences will inspire and motivate several such local initiatives in resource-limited settings.

The "Vision and Change in Undergraduate Biology Education: A Call to Change" document has recommendations about utilizing innovative pedagogy and the integration of authentic research experiences into individual courses and biology programs overall to ensure that all undergraduates can experience the processes, nature, and limits of science. However, there are several undergraduate institutions with limited resources that are unlikely to provide such experiences to their students. Our Perspective article showcases a collaborative model where a research institute collaborated with two teaching colleges to devise and implement a small program that was effective in providing an authentic research experience to undergraduate students that can be sustainable. The model can be used by other undergraduate institutions with either perceived or real resource limitations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Committees of Bhavan's Vivekananda College and St.Ann's College for Women. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AKC designed the programs and study. YA and KA contributed equally to the implementation of the program. YA, KA, ChJ, KSM, and AKC contributed toward preparation of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.589405/full#supplementary-material

Supplementary Figures 1–3 | Summary of student responses to the questionnaire. The left panels (in cream box) represents data from the control group (n = 102). The second and third columns correspond to responses we obtained from students who participated in the "Exploring Microbial Diversity" workshop (41/45 students completed the questionnaire, 91% response rate); second column corresponds to student/workshop participants experiences in regular college courses, while the third column corresponds to their experiences in the workshop. All data are represented as percentages. Student responses relating to emphasis on memorization, analysis, synthesis, and application (**Supplementary Figure 1**); impact of college courses or workshop on independent thinking, analysis (beyond memorization), active engagement, enhancement of academic interest, and academic autonomy (**Supplementary Figure 2**); student involvement with content (**Supplementary Figure 3**).

REFERENCES

Alberts, B. (2005). A wakeup call for science faculty. *Cell* 123, 739–741. doi: 10.1016/j.cell.2005.11.014

Basalla, J., Harris, R., Burgess, E., Zeedyk, N., and Wildschutte, H. (2020). Expanding Tiny Earth to genomics: a bioinformatics approach for an undergraduate class to characterize antagonistic strains. *FEMS Microbiol. Lett.* 367, doi: 10.1093/femsle/fnaa018

Bentley, A. M., Artavanis-Tsakonas, S., and Stanford, J. S. (2008). Nanocourses: a short course format as an educational tool in a biological sciences graduate curriculum. *CBE—Life Sci. Educ.* 7, 175–183. doi: 10.1187/cbe.07-07-0049

Ferren, A. S., and Anderson, C. B. (2016). Integrative learning: making liberal education purposeful, personal, and practical. *New Dir. Teach. Learn.* 2016, 33–40. doi: 10.1002/tl.20172

Frank, J. A., Reich, C. I., Sharma, S., Weisbaum, J. S., Wilson, B. A., and Olsen, G. J. (2008). Critical Evaluation of Two Primers Commonly Used for Amplification of Bacterial 16S rRNA Genes. *Appl. Environ. Microbiol.* 74, 2461–2470. doi: 10.1128/AEM.02272-07

Frantz, K. J., Demetrikopoulos, M. K., Britner, S. L., Carruth, L. L., Williams, B. A., Pecore, J. L., et al. (2017). A Comparison of Internal dispositions and career trajectories after collaborative versus apprenticed research experiences for undergraduates. *CBE Life Sci. Educ.* 16:206. doi: 10.1187/cbe.16-06-0206

Government of India and University Grants Commission [UGC] (2019). *University Grants Commission: Annual Reports/ Annual Accounts, 2018–19*. Available online at: https://www.ugc.ac.in/page/Annual-Report.aspx (accessed July 26, 2020).

Hatcher, J. A. (2011). Assessing civic knowledge and engagement. *New Dir. Institutional Res.* 2011, 81–92. doi: 10.1002/ir.382

Henter, H. J., Imondi, R., James, K., Spencer, D., and Steinke, D. (2016). DNA barcoding in diverse educational settings: five case studies. *Philos. Trans. R. Soc. B Biol. Sci.* 371:20150340. doi: 10.1098/rstb.2015.0340

Jordan, T. C., Burnett, S. H., Carson, S., Caruso, S. M., Clase, K., DeJong, R. J., et al. (2014). A broadly implementable research course in phage discovery and genomics for first-year undergraduate students. *mBio* 5:e01051-13. doi: 10.1128/mBio.01051-13

Lakhotia, S. C., Shashidha, L. S., and Vale, R. (2013). Excellence in science education and research. *Curr. Sci.* 104, 163–165.

Linn, M. C., Palmer, E., Baranger, A., Gerard, E., and Stone, E. (2015). Undergraduate research experiences: Impacts and opportunities. *Science* 347:1261757. doi: 10.1126/science.1261757

Association of American Colleges and Universities (2007). *College Learningfor the New Global Century A Report from the National Leadership Council for Liberal Education & America's Promise*. Washington, DC: Association of American Colleges and Universities.

Penn, J. D. (2011). The case for assessing complex general education student learning outcomes. *New Dir. Institutional Res.* 2011, 5–14. doi: 10.1002/ir.376

Saberwal, G. (2019). Will our education system enable India to be a super-power any time soon? *Curr. Sci.* 116, 509–510.

Schütte, U. M. E., Abdo, Z., Bent, S. J., Shyu, C., Williams, C. J., Pierson, J. D., et al. (2008). Advances in the use of terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes to characterize microbial communities. *Appl. Microbiol. Biotechnol.* 80, 365–380. doi: 10.1007/s00253-008-1565-4

Sieg, R. D., Narayanan, M., Sabatini, J., Beverly, N., Surendran, G., and Smyth, D. S. (2019). Incubating the SENCER ideals with project-based learning and undergraduate research: perspectives from two liberal arts institutions. *Sci. Educ. Civ. Engagem.* 11, 50–63.

Staub, N. L., Poxleitner, M., Braley, A., Smith-Flores, H., Pribbenow, C. M., Jaworski, L., et al. (2016). Scaling up: adapting a phage-hunting course to increase participation of first-year students in research. *CBE—Life Sci. Educ.* 15:ar13. doi: 10.1187/cbe.15-10-0211

Wei, C. A., and Woodin, T. (2011). Undergraduate research experiences in biology: alternatives to the apprenticeship model. *CBE—Life Sci. Educ.* 10, 123–131. doi: 10.1187/cbe.11-03-0028

Zai, R. (2015). Reframing general education. *J. Gen. Educ.* 64, 196–217. doi: 10.5325/jgeneeduc.64.3.0196

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Loop-Mediated Isothermal Amplification (LAMP) as a Rapid, Affordable and Effective Tool to Involve Students in Undergraduate Research

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Undergraduate research (UR) is a high-impact practice (HIP) to engage undergraduate student in science, technology, engineering and mathematics (STEM), especially from underrepresented groups. UR experiences (UREs) can be integrated into the classroom, making authentic research experiences inclusive and available to all students. However, developing UR pedagogy can be challenging for faculty in resource-limited labs, such as community colleges and small liberal arts colleges. Often molecular biology research methods are expensive, time-consuming and need equipment not readily available or affordable in small schools. Polymerase chain reaction (PCR) is one of the most commonly used techniques in research labs and many UREs. We have investigated loop-mediated isothermal amplification (LAMP) as an inexpensive, accessible alternative to PCR for DNA amplification enabling the identification of microorganisms in the context of UREs. LAMP does not require expensive instrumentation or reagents and uses equipment commonly found in teaching labs. By performing the technique, students learn several key scientific skills that will be useful in their undergraduate or graduate STEM careers. We designed guided independent research experiences for several undergraduates that included the use of LAMP. Students successfully applied the technique to culture samples of common environmental bacteria, including *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and *Enterococcus*, and were in addition, able to detect both *Salmonella* and *Enterococcus* in directly sampled environmental waters. To highlight the accessibility and affordability of this URE, a simple boiling method was used for DNA preparation from environmental samples. Student response

data show positive attitudes toward UR when LAMP is utilized as a research tool to tackle relevant biological questions. The feasibility of using simplified LAMP in UREs points to a potential, more expanded application to public engagement with science and broader and more inclusive interactions with the research community.

Keywords: LAMP, research tool, undergraduate research (UR), HIP's, stem education

INTRODUCTION

The Association of American Colleges and Universities has recognized several instructional modalities as high-impact practices (HIPs) that increase student retention rates and student engagement (Kuh, 2008). Among these practices, undergraduate research (UR) has been found to be a particularly effective pedagogy for the engagement and retention of undergraduate students (Kuh, 2008). UR can provide students with not only scientific skills but also increased self-confidence, improved oral and written presentation skills and enhanced critical thinking (Kuh and O'Donnell, 2013).

Two main challenges are faced when creating UR experiences (UREs). First, it may be difficult to design projects that are realistically feasible and that also engage students in relevant authentic queries. Second, many procedures require reagents, equipment and/or central technical support that are beyond the means of many undergraduate institutions. One category of studies that can address the first issue consists of projects that focus on environmental sampling, such as the identification of pathogens from environmental samples like soil and water. The current COVID-19 pandemic has heightened student awareness of the value of such studies. However, environmental pathogen identification typically involves the use of polymerase chain reaction (PCR) for DNA amplification, which is time consuming, expensive and can be difficult for students to perform accurately. At Queensborough Community College (QCC) we have developed UREs in which loop-mediated isothermal amplification (LAMP) can be used as a simpler, sensitive and far less expensive alternative to PCR for the identification of microbes in samples obtained from locations on campus as well as from the surrounding urban environment. While comparable in its value as a teaching tool to PCR, LAMP is over tenfold less expensive, as well as more sensitive and more robust in handling complex biological samples (Kaneko et al., 2007; Mori and Notomi, 2009; Law et al., 2014; Warghane et al., 2017). The simplicity of colorimetric readouts, coupled with the simplicity of the amplification procedure itself, makes it easy to incorporate LAMP in a classroom context, so that the method can be used to support course-based UREs (CUREs) as well as independent UR projects.

The standard LAMP assay does not require equipment beyond those that are available in standard biology laboratories (see **Supplementary Figure S1**). The simplicity of the setup derives from the dependence of LAMP on the inherent strand-displacing activity of the Bst polymerase, avoiding the need for the repeated high-temperature intervals employed in PCR, so that the entire reaction can be performed at a single temperature (60–68°C), using a non-denatured double-stranded DNA template (see **Supplementary Figure S2**). However, sample preparation methods prior to the assay may still consume classroom time and require specialized equipment and/or expensive supplies. By using the simple boiling method of sample preparation, LAMP can be made accessible not only to on-campus undergraduates but also to the at-home student in the context of distance learning, now emerging as a dominant mode of instruction during the COVID-19 pandemic. Even beyond the at-home student, we suggest that simplified LAMP protocols that avoid or minimize sample preparation may be uniquely positioned to promote a broader scientific engagement with lay communities and foster enhanced interactions between the research community and the public.

We describe here some initial illustrative data generated by several of our undergraduates with whom we designed guided UREs. These students were able to use LAMP to detect several bacterial microorganisms commonly found in the environment: *Escherichia coli (E. coli)*, *Salmonella* spp. (*S.* spp.), *Staphylococcus aureus (S. aureus)*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Enterococcus* (Hill et al., 2008; Tang et al., 2012; Wang et al., 2015; Lin et al., 2017; Hu et al., 2018).

MATERIALS AND METHODS

Setting the URE

The students who participated part in the URE were either Biotechnology or Chemistry majors in an urban community college who had enrolled in a research laboratory internship course. Students had taken two semesters of introductory biology as well as biotechnology either prior to or concurrent with the URE.

Bacterial Strains and Growth Media

Escherichia coli, *Salmonella* spp., *Staphylococcus aureus*, *Enterococcus faecalis*, and methicillin-resistant *Staphylococcus aureus* (MRSA) were grown in Brain Heart Infusion Broth or Tryptic Soy Broth.

Sample Preparation

Soil samples (approximately 5 ml) were suspended in 50 ml water in conical tubes and allowed to settle prior to removal of 10 ml supernatant for DNA extraction by the boiling method (De Medici et al., 2003). DNA extraction from standing water samples (10 ml) was carried out by the boiling method.

TABLE 1 | Target genes and protocols for LAMP assays.

	Target gene	Incubation temperature	References
E. coli	*malB*	66°C	Hill et al. (2008)
Salmonella spp.	*fimY*	65°C	Tang et al. (2012)
Salmonella enteritidis	*safA*	65°C	Azinheiro et al. (2018)
S. aureus	*nucAfemA* (MRSA)	60°C60°C	Wang et al. (2015)Lin et al. (2017)Chen et al. (2017)
Enterococcus spp.	23S rRNA	64°C	Martzy et al. (2017)

For *Enterococcus* tests, samples (10 ml) were collected from the East River (40.80557°N, 73.79661°W) and extracted by the boiling method.

DNA preparation was either by column-based purification (Quick-DNA Microprep Kit, Zymo Research, Irvine, CA, United States) or by the boiling method (De Medici et al., 2003). For the boiling method, all samples were first spun and resuspended at $1/10^{th}$ original volume.

LAMP Assay

The target genes and protocols employed in the study are summarized in **Table 1**. Note that *fimY* (encodes for the Fimbriae Y protein) and *nucA* (encodes for the nuclease) are conserved targets for all serotypes of *Salmonella* and *S. aureus*, respectively, while *safA* (encodes for the major subunit of *S. enterica* atypical fimbriae) is specific for *Salmonella enteritidis*. *malB* (encodes for maltose operon protein B) is specific to *E. coli* and *femA* (encodes for protein that affect the level of methicillin resistance) is specific for methicillin-resistant *S. aureus* (MRSA).

All LAMP incubations were for 1 hour. Reaction products were visualized by ethidium bromide staining of agarose gels. The amplified products from the LAMP reaction are not single-size amplicons but rather exhibit a range of different product sizes (**Supplementary Figure S2**, Part 5b). Thus, a positive LAMP reaction appears as a smear or a ladder of amplified products on an agarose gel, rather than a single band as seen with PCR (Hill et al., 2008; Tang et al., 2012; Wang et al., 2015; Lin et al., 2017; Hu et al., 2018). For detection of *Enterococcus* spp. in environmental samples, the LAMP amplification of the 23S rRNA target was performed with the addition of SYBR Green I, and the reaction was performed for 60 min in a qPCR machine (Quantstudio 6, Applied Biosystems in order to quantitate fluorescence each minute as a measure of product yield).

Detection of *Enterococcus* by Enterolert

The Enterolert test (IDEXX Laboratories, Westbrook, Maine) is an enzymatic reaction based on the ability of *Enterococcus* beta-glucosidase to cleave 4-methyl-umbelliferyl b-D-glucoside, yielding a fluorescent product. Briefly, water samples for enumeration of *Enteroccoci* via IDEXX Enterolert Media were collected from the East River (40.80557°N, 73.79661°W) in 1 L acid washed high-density polyethylene bottles. Samples were processed the same day per the manufacturer's protocol. After incubation at 41°C for 24 h, samples were read using a 6-watt, 365 nm UV light box, and enumerated using the IDEXX MPN table.

Laboratory Safety

The URE in which students participated includes education in the biology and clinical impact of the pathogens studied. Standard biosafety practices for microbiology laboratory work were enforced, including adherence to Biosafety Level 2 (BSL2) procedures. Aseptic technique with a Bunsen burner was used for bacterial work. No enrichments of environmental samples were performed, although students were made aware that these samples can contain disease-causing organisms.

RESULTS

Amplification From DNA of Known Strains

Optimization

As a first step toward the incorporation of LAMP in an URE, students performed amplifications using column-purified DNA prepared from cultures of *E. coli*, *S. aureus*, *Salmonella* spp., and *Enterococcus* spp. As shown in **Figures 1**–**4**, successful amplification was obtained with all four strains. The *E. coli* and *S. aureus* amplifications were used as a basis for optimization studies, and students were able to demonstrate a range of product

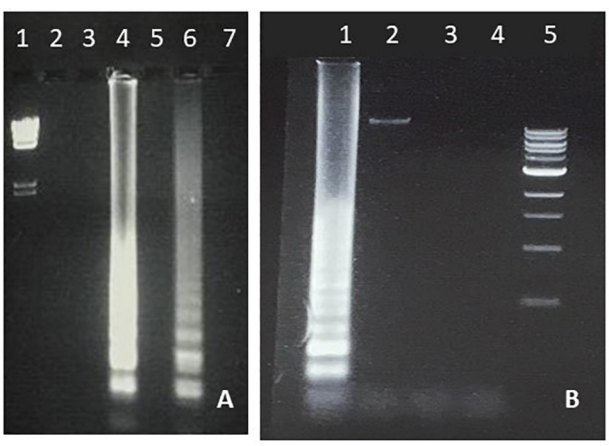

FIGURE 1 | LAMP amplification of *E. coli.* **(A)** Temperature optimization of *E. coli* LAMP. Lane 1, lambda *Hin*dIII marker. Lanes 2, 4, and 6 were generated with chromosomal DNA and Lanes 3, 5, and 7 with water as negative control. Lanes 2 and 3 were incubated at 70°C, Lanes 4 and 5 at 66°C and Lanes 6 and 7 at 62°C. **(B)** Specificity of *E. coli* LAMP. Lane 1; *E. coli*, Lane 2; *S. typhimurium*, Lane 3; *Staphylococcus aureus*, Lane 4; water, Lane 5; 1 Kb marker. All reactions were carried out at 66°C.

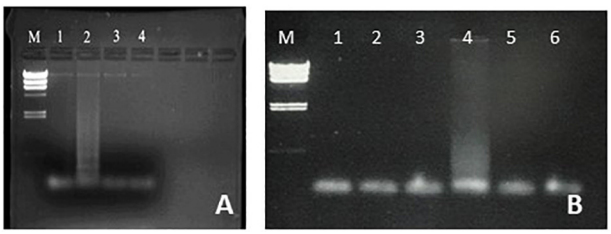

FIGURE 2 | Optimization of *S. aureus* LAMP amplification. **(A)** Optimization of LAMP targeting the *femA* gene. Lane M, lambda *Hin*dIII marker. Reactions carried out at 55°C (lane 1), 60°C (lane 2), 65°C (lane 3), and 70°C (lane 4). **(B)** Optimization of LAMP targeting the *nuc* gene. Lane M, lambda *Hin*dIII marker. Lanes 2, 4, and 6 have *S. aureus* template and lanes 1, 3 and 5 are negative controls with water in place of template. Lanes 1, 2: 57°C. Lanes 3, 4: 62°C. Lanes 5, 6: 67°C.

yields by temperature variation, with optimal results within the 60–66°C range as expected (**Figures 1**, **2**). LAMP affords multiple additional parameters for student design of optimization studies, including inner/outer primer ratio, magnesium concentration and incubation time, which will be incorporated in future development of the URE.

Specificity

Students were able to confirm the specificity of LAMP for *E. coli* (**Figure 1**), *Salmonella* spp. (**Figure 3**), and *Enterococcus* (**Figure 4**), as well as the conservation of the *fimY* target in multiple *Salmonella* strains (**Figure 3**). Students were also able to observe non-specific elements, such as primer bands and chromosomal DNA (**Figure 1B**, lane 2). An ambiguous result was obtained with *Streptococcus* (*Enterococcus*) (**Figure 3A**, lane 5), likely reflecting contamination and/or excess chromosomal DNA. The exquisite sensitivity of LAMP enhances the opportunity for students to observe such false positives, providing opportunity for critical thinking and troubleshooting.

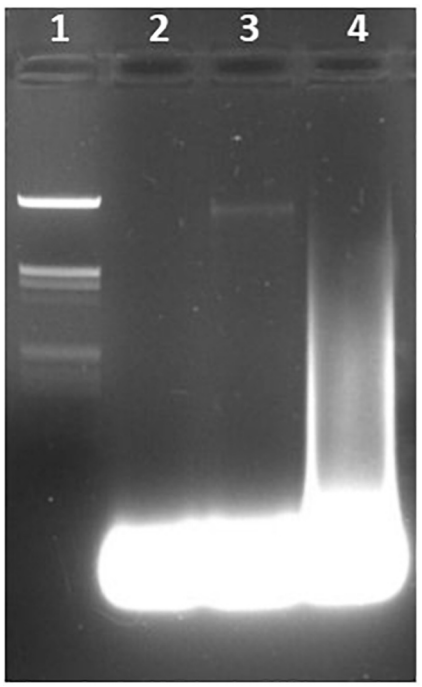

FIGURE 4 | Detection of the 23S rRNA gene of *Enterococcus faecalis* using LAMP. Lane 1, lambda *Hin*dIII marker. Lane 2, water (negative control). Lane 3, *E. coli*. Lane 4, *E. faecalis* DNA.

DNA-Preparation Method

To assess the dispensability of column-based DNA preparation in the pedagogical use of LAMP, students were given a blind series of sixteen distinct *S. aureus* strains, of which six were MRSA, and used both column and boiling methods for each strain, prior to performing *mecA* LAMP to identify MRSA strains. Similar performance was observed with the two DNA preparation methods: in each case, two MRSA

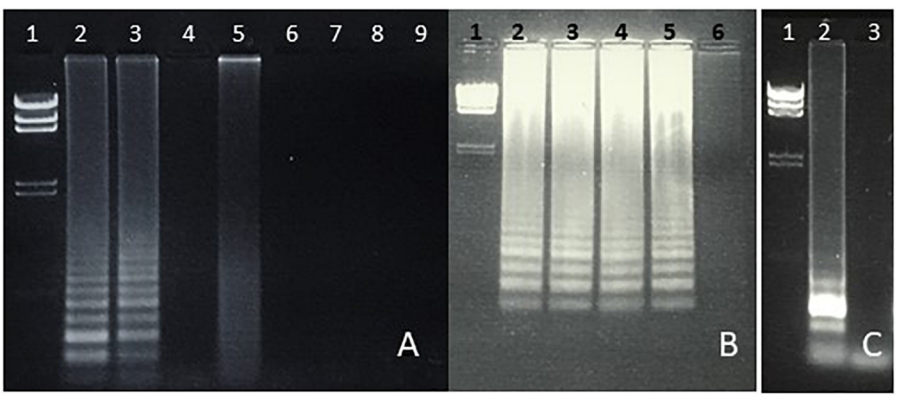

FIGURE 3 | (**A, B)** Specificity of LAMP amplification of *Salmonella* spp. (*fimY* target). **(A)** Lane 1, lambda *Hin*dIII marker. Lane 2, *S. typhimurium*. Lane 3, *S. Newport*. Lane 4, *E. coli*. Lane 5. *Enterococcus* (*Streptococcus) faecalis.* Lane 6, *S. aureus.* Lane 7, *Proteus vulgaris*. Lane 8, *Serratia marcescens*. Lane 9, water (negative control). **(B)** Lane 1, lambda *Hin*dIII marker. Lane 2, *S. typhimurium*. Lane 3, *S. heidelberg*. Lane 4, *S. enteritidis*. Lane 5, *S. Newport*. Lane 6, water (negative control). **(C)** LAMP amplification of *S. enteritidis* (*safA* target). Lane 1, Lambda *Hin*dIII marker. Lane 2, *S. enteritidis*. Lane 3, water.

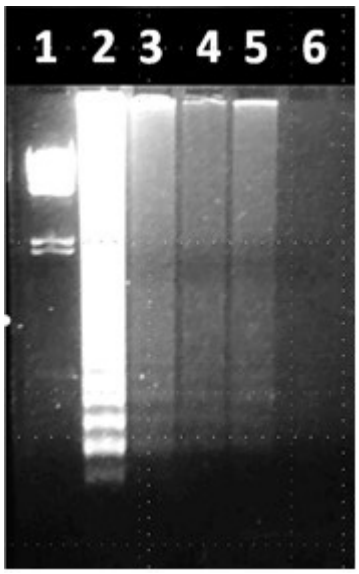

FIGURE 5 | Use of LAMP to detect *Salmonella* spp. in environmental samples (*fimY* target). Lane 1, lambda *Hin*dIII marker. Lane 2, water spiked with *S. typhimurium* (positive control). Lane 3, Alley Pond water. Lane 4, standing water in the QCC parking lot. Lane 5, soil at QCC resuspended in water. Lane 6, distilled water (negative control).

strains were correctly identified (one of which was identified by both methods) and in each case there were four false negatives (data not shown). Overall, these data are supportive of the utilization of LAMP in classroom-based and distance learning settings.

Amplification From Environmental Samples

Our students used the LAMP assays they had established to assess environmental samples for either *Salmonella* spp. or *Enterococcus* spp. Enrichments were not performed, since the goal was to determine whether LAMP assay was adequate to allow students to detect these microorganisms with minimal processing. *Salmonella* spp. was detected in a water sample from a kettle pond in an urban park (Alley Pond, Bayside, NY, United States), as well as in standing water and soil samples within the college campus (**Figure 5**). *Enterococcus* was assessed by LAMP in a series of samples taken over several months from three locations in an urban waterway (East River in New York City). *Enterococcus* levels in these samples were also quantitatively assessed by defined substrate technology (Enterolert; IDEXX). To allow the students to more objectively relate the Enterolert data to their qualitative LAMP findings, the LAMP results were made semi-quantitative by determining the reaction time (T_t) required to achieve a threshold level of SYBR Green fluorescence. The T_t values were used to divide the samples into a LAMP-High group ($T_t < 22$) and a LAMP-Low group ($T_t > 30$). The samples were also divided on the basis of the Enterolert data into groups that were either high or low for most probable number (MPN, comparable to CFU). The MPN-High group had MPN values of 110–3,578) while the MPN-Low group had MPN <10–30. It was observed that seven of eight

Sample	Date	MPN	LAMP
IT1	5/23/2019	3578	High
HC1	5/23/2019	<10	High
MAR1	5/23/2019	354	High
IT2	5/30/2019	1515	High
HC2	5/30/2019	2282	High
MAR2	5/30/2019	145	High
IT3	6/6/2019	20	Low
HC3	6/6/2019	10	High
MAR3	6/6/2019	10	Low
IT4	6/13/2019	402	Low
HC4	6/13/2019	1565	High
MAR4	6/13/2019	10	Low
IT5	6/20/2019	20	High
HC5	6/20/2019	30	Low
MAR5	6/20/2019	110	High
IT6	6/27/2019	<10	Low
HC6	6/27/2019	20	Low
MAR6	6/27/2019	10	Low

Samples reordered by MPN value

	Sample	MPN	LAMP
MPN-High	IT1	3578	High
	HC2	2282	High
	HC4	1565	High
	IT2	1515	High
	IT4	402	Low
	MAR1	354	High
	MAR2	145	High
	MAR5	110	High
MPN-Low	HC5	30	Low
	IT3	20	Low
	IT5	20	High
	HC6	20	Low
	HC3	10	High
	MAR3	10	Low
	MAR4	10	Low
	MAR6	10	Low
	HC1	<10	High
	IT6	<10	Low

p = 0.02

FIGURE 6 | Assessing *Enterococcus* spp. in water samples using LAMP. Water samples were collected at the indicated times from three different locations in the East River in New York City. Samples were tested for *Enterococcus* spp. both by Enterolert (IDEXX) and LAMP. LAMP results were made semi-quantitative by kinetic monitoring of SYBR Green fluorescence (see text). The association of high LAMP values with high MPN values was assessed by one-tailed Fisher's exact test.

TABLE 2 | Materials required to set up LAMP and PCR.

	Power supply ($600)	Electrophoresis tank ($400)	UV transilluminator ($1,000–5,000)	Thermocycler ($3,000)	Water bath ($500)	Total cost
PCR	✓	✓	✓	✓	–	$5,500
LAMP	✓	✓	✓	–	✓	$2,500
Simple LAMP*	–	–	–	–	✓	$500
At-home LAMP*	–	–	–	–	–	$0

**LAMP using colorimetric readout and samples prepared by boiling.*

MPN-High samples were LAMP-High, compared to three of ten samples in the MPN-Low group. This difference was significant at $p = 0.02$ by Fisher's exact text (**Figure 6**). These results show that students are able to apply LAMP semi-quantitatively to assess environmental levels of bacterial pathogens as part of an URE.

DISCUSSION

We sought a new approach to developing UR projects to identify common microorganisms that can be detected in environmental samples. Standard methods for the detection of these microorganisms involve culturing and isolating the bacteria, which is a time-consuming and laborious process. Amplification of genomic DNA by PCR is considerably faster but requires an expensive thermocycler and somewhat expensive reagents. We propose LAMP as a promising alternative method to detect microorganisms, as it is rapid, inexpensive with respect to both equipment and reagents, and does not require much effort to set up (**Tables 2**, **3** and **Supplementary Figure S1**).

We demonstrate here that, even in the undergraduate setting, the LAMP technique can be effectively used to replace PCR to help identify bacterial microorganisms. Our data demonstrate that students with modest training in molecular biology can be involved in authentic research aimed at detecting microbes in unknown environmental samples. We have trained eleven students over the course of three semesters to successfully use this technique to detect environmental microbes. We used LAMP to amplify four common and clinically relevant environmental bacteria: *E. coli*, *S. enteritidis*, *Enterococcus*, and *S. aureus*. All of the data presented here were generated in student-performed studies conducted within a semester time frame, as part of an independent study research course. The students not only acquired basic laboratory proficiency but were able to engage the primary literature.

Two of our student researchers went on to present their findings at regional and national conferences and won prizes for their posters. One student went on to obtain a Research Experiences for Undergraduates (REU) internship in a prestigious 4-year college. Our preliminary data illustrate the abundant opportunities provided by LAMP technology for students to develop skills in critical thinking and experimental design. Students can optimize multiple parameters, troubleshoot unexpected or discrepant findings, develop comparisons with alternative assays, and test hypotheses with respect to the incidence and distribution of multiple microbial microorganisms. A student response survey data shows that majority of the students had a positive and engaging UR experience (**Figure 7**).

TABLE 3 | Reagent cost comparison between LAMP, PCR, and qPCR.

	Real time PCR (per reaction)	Traditional PCR (per reaction)	LAMP (per reaction)
Enzyme (*Taq* or *Bst*)	$2.50	-	-
SYBR™ Green PCR Master mix 1 ml = $125 (ThermoFisher)		$0.47 per Unit	- $0.07
Taq Polymerase 500 Unit (U) = $236 0.25–2.5 U/50 μl reaction (ThermoFisher)			
Bst Enzyme 8,000 U/ml = $70 (New England Biolabs)			
Plates or Strip 300 strips = $126	$0.42/strip $0.0525/well	–	–
Microfuge tubes (ThermoFisher) 500 = $22	$0.044	$0.044	$0.044
PCR tubes 1,000 = $119 (ThermoFisher)	–	$0.12	–
Primers: (0.1–1 μM) 100 μM–$40	Forward and Backward $0.04	Forward and Backward $0.04	F3/B3 = $0.04 LF/FB = $0.04 FIP/BIP = $0.08
dNTP's 100 mM 4 × 250 μl = $320 (ThermoFisher)		50 μM each 2,000 reactions $0.16	50 μM each 2,000 reactions $0.16
Agarose gel (ThermoFisher) 100 g = $145	1% gel–$1.45 $0.08 per lane	1% gel–$1.45 $0.08 per lane	1% gel–$1.45 $0.08 per lane
TOTAL*	$2.71¥	$0.91[8]	$0.52[8]

**This is an underestimate as plastic tips/DNA markers/buffers are not calculated.*
¥Most real time PCR reactions are run in triplicate with appropriate internal controls.
[8]Samples can be run in duplicate.
The estimate here is within reported data for the cost per LAMP reaction to be approximately 60–70 cents (1) while most DNA amplification cost between $1–2 per reaction and $5–7 per reaction for real time PCR (2).

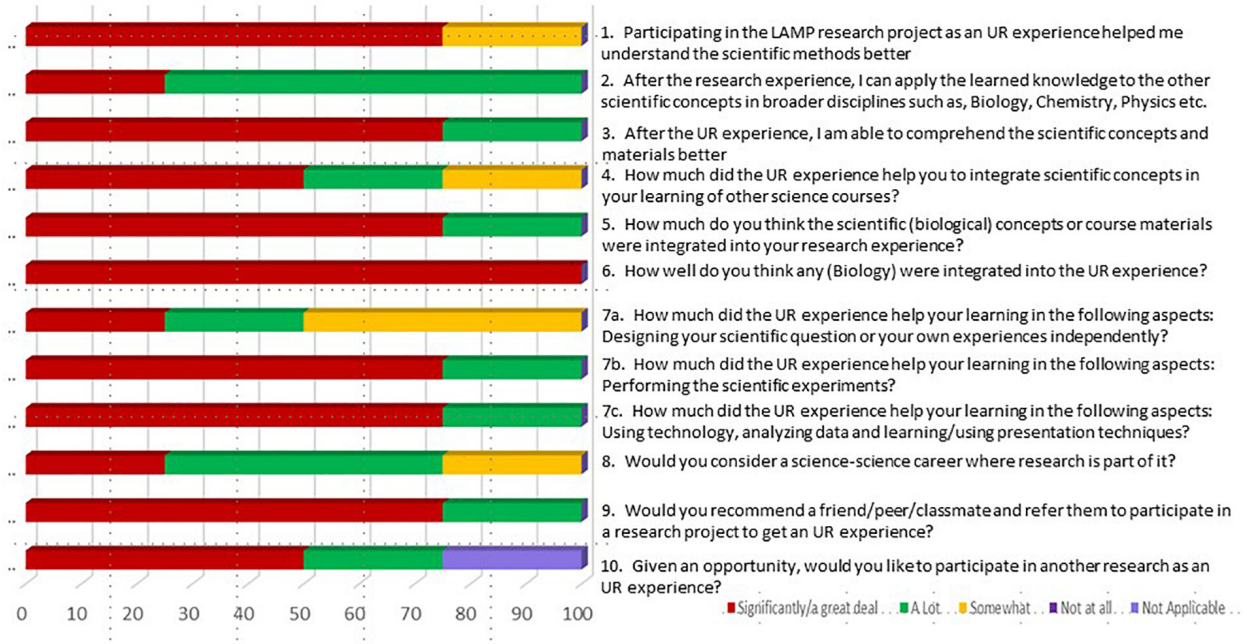

FIGURE 7 | Survey of student attitudes. An anonymous survey was conducted using Google forms.

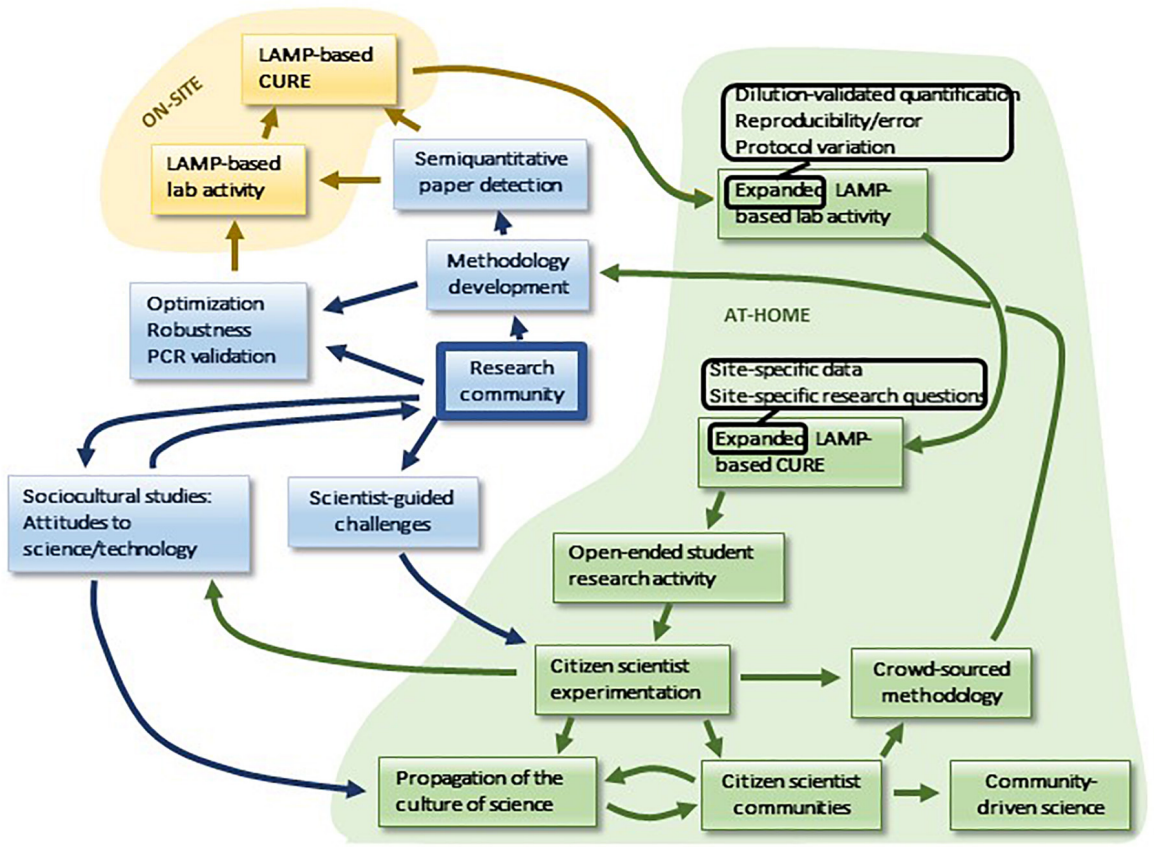

FIGURE 8 | A potential usage of LAMP in distance learning.

Loop-mediated isothermal amplification technology shares with PCR a notable potential to connect URE with important public health issues. Since *E. coli*, *Salmonella* spp., *Enterococcus* spp., and *S. aureus* spread via contaminated food or water, as well as via infected individuals, environmental detection of these microorganisms can limit the occurrence and magnitude of outbreaks (Curran, 2017). Notably, LAMP has emerged as a relevant technology for the detection of norovirus, *Clostridium perfringens*, and *Campylobacter jejuni*, three of the five most prevalent foodborne pathogens in the United States (Fukuda et al., 2006; Yamazaki et al., 2009; Kaneko et al., 2011), and has recently been used to detect COVID-19 (Kashir and Yaqinuddin, 2020). Students could use LAMP to detect these and other types of microorganisms in the course of developing their own projects. The only additional reagents required would be new primers.

Incorporating authentic research in undergraduate curriculum is challenging due to technical demands and time constraints (Smyth, 2017). Implementing LAMP-based research projects as a CURE is a potential solution. Our data show that both cultures and environmental samples can be effectively processed for LAMP by simple boiling, which should greatly facilitate the design of these experiences in a classroom setting. We intend to implement LAMP-based CUREs in several major and non-major Biology courses using environmental samples. Students will be encouraged to formulate hypotheses as to which environmental sites or site categories are likely to harbor the greatest prevalence of pathogenic microorganisms. Faculty mentors will assist by engaging students in learning about microbes in the context of urban ecology and public health. Pre/post surveys will be used to assess impact on student understanding of scientific method and microbial biology, as well as attitudes toward potential STEM careers.

Finally, a unique feature of LAMP is the potential for its use in distance learning, and more broadly for both student- and community-based research activity, presenting an unusual opportunity for the expansion of public engagement in science as well as interaction with the research community. This potential developmental sequence is summarized in **Figure 8**. In the context of distance learning, LAMP is uniquely suited to at-home use in a manner that parallels on-site procedures. All components are stable, including the *Bst* polymerase, which displays remarkable stability (Meridian Bioscience). Any household with an oven, a pot and a thermometer can establish the necessary constant-temperature incubation conditions, and colorimetric readout is readily accomplished. The boiling method for DNA prep can be readily adapted for at-home implementation: inexpensive oven-safe evaporating dishes can be used for sample concentration, substituting for centrifugation. Non-specific products are expected to be rare due to the requirement for hybridization at six independent sequences in the target. Furthermore, it is likely that paper strip-based readout methods will become available that will facilitate semiquantitative analysis (Hongwarittorrn et al., 2017). Quantitation by such methods, as well as by dilution, will allow at-home students to pursue expanded LAMP-based lab activities, including error analysis and protocol variations, which take advantage of the time and flexibility afforded by flipped lab designs. Similarly, at-home CUREs offer expanded possibilities in comparison to on-site research, as students can develop research questions and collect samples in a manner that is tailored to the unique as well as shared aspects of their individual locations. Continual access to the home "laboratory" is likely to encourage students to generate additional research projects in an open-ended manner, providing an unusual opportunity for training in project design at the undergraduate level.

Moreover, the possibilities for at-home LAMP are not limited to the student body, which may serve as a vanguard for the encouragement of similar scientific research activity in communities. Therein potentially lies a unique opportunity for the development of the public culture of science. For example, while it is becoming increasingly common for scientists to crowdsource sample acquisition from the lay public, LAMP is perhaps the unique technology that may permit lay crowdsourcing, not only of samples, but also of methodological data. Lay LAMP-tinkerers may make a contribution to the professional research community, if their numbers outweigh their lack of scientific sophistication. Scientists may contribute to and help guide this process by offering research challenges to such lay communities. Communities of citizen scientists can potentially organize projects of their own, and they may also be a source of sociocultural data for the professional study of the culture of science (**Figure 8**). By engaging the public this way, we anticipate that this may increase understanding and engagement with science as well as broadening access to and increasing inclusiveness in the process of science.

In summary, incorporation of LAMP in URE has many benefits, including low cost, speed, ease of training, and the ability to engage undergraduates in meaningful UREs. Moreover, beyond these technical advantages, LAMP has unusual promise as a technology for expanding student and community experience in authentic scientific inquiry. UR has become a hallmark national trend as a component of HIP pedagogy. We have been able to observe the value of UR to undergraduate education on a personal level, noting the growth in self-confidence, independence and communication skills in our students as they progress through the program. Further development of LAMP-based approaches to UR will allow students to gain first-hand knowledge of STEM careers, and more broadly to apply newly acquired skills and experience to their further education and professional development.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

All authors contributed to the design of the experiments and agreed to working on different projects. AN planned the project, supervised, and wrote an initial draft. AO revised and rewrote the manuscript, analyzed data, and added a figure.

DS and MT contributed to the revision. AN, AO, and MT designed and implemented IRB-approved student survey. CP and MK provided discussion and strategic approach to LAMP research regarding *Enterococcus* and *Salmonella*, respectively. All the students (KP, GL, DM, OB, MF, JS, WK, EC, YK, TN, MF, JE, and MM) performed experiments, data analysis and participated in manuscript preparation. All authors critically discussed and contributed to the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.603381/full#supplementary-material

Supplementary Figure 1 | Comparison of DNA amplification using PCR **(A)** or LAMP **(B)**. PCR requires the use of a thermocycler and electrophoresis equipment **(A)**. In LAMP, self-priming obviates the need for a thermocycler, and colorimetric readout provides an alternative to electrophoresis **(B)**.

Supplementary Figure 2 | Schematic diagram of LAMP. *Part 1.* Template DNA with primer target sequences indicated (F3c, F2c, and F1c). *Part 2.* Primer FIP binds to target sequence F2c, with a 5′ overhang containing F1c (top panel). Primer F3 is used to unzip and release strand A2 (middle panel), which then forms a loop at its 5′ end via F1c-F1 complementarity (bottom panel). *Part 3.* The 3′ end of strand A2 is targeted by primer BIP, followed by unzipping with primer B3 to release strand A4, which can forms loops at both 5′ and 3′ ends. *Part 4.* The resulting dumbbell (strand A4) is comparable to a similar dumbbell (strand B4) formed by a corresponding series of events (not shown) beginning with targeting of B2c in strand B (*Part 1*). *Part 5.* Loop amplification yields concatemers of target sequences, beginning either with strand A4 (*Part 5a*) or with strand B4 (*Part 5b*).

REFERENCES

Azinheiro, S., Carvalho, J., Prado, M., and Garrido-Maestu, A. (2018). Evaluation of different genetic targets for *Salmonella enterica* Serovar Enteritidis and Typhimurium, using loop-mediated isothermal amplification for detection in food samples. *Front. Sustain Food Syst.* 2:5. doi: 10.3389/fsufs.2018.00005

Chen, C., Zhao, Q., Guo, J., Li, Y., and Chen, Q. (2017). Identification of Methicillin-Resistant Staphylococcus aureus (MRSA) Using Simultaneous Detection of mecA, nuc, and femB by Loop-Mediated Isothermal Amplification (LAMP). *Curr. Microbiol.* 74, 965–971. doi: 10.1007/s00284-017-1274-2

Curran, E. (2017). Infection outbreaks in care homes: prevention and management. *Nurs. Times* 113, 18–21.

De Medici, D., Croci, L., Delibato, E., Pasquale, S., Filetici, E., and Laura, T. (2003). Evaluation of DNA extraction methods for use in combination with SYBR Green I real-time PCR to detect *Salmonella enterica* Serotype Enteritidis in poultry. *Appl. Environ. Microbiol.* 69, 3456–3461. doi: 10.1128/aem.69.6.3456-3461.2003

Fukuda, S., Takao, S., Kuwayama, M., Shimazu, Y., and Miyazaki, K. (2006). Rapid detection of norovirus from fecal specimens by real-time reverse transcription-loop-mediated isothermal amplification assay. *J. Clin. Microbiol.* 44, 1376–1381. doi: 10.1128/jcm.44.4.1376-1381.2006

Hill, J., Beriwal, S., Chandra, I., Paul, V. K., Kapil, A., Singh, T., et al. (2008). Loop-mediated isothermal amplification assay for rapid detection of common strains of *Escherichia coli*. *J. Clin. Microbiol.* 46, 2800–2804. doi: 10.1128/jcm.00152-08

Hongwarittorrn, I., Chaichanawongsaroj, N., and Laiwattanapaisal, W. (2017). Semi-quantitative visual detection of loop mediated isothermal amplification (LAMP)-generated DNA by distance-based measurement on a paper device. *Talanta* 175, 135–142. doi: 10.1016/j.talanta.2017.07.019

Hu, L., Ma, L. M., Zheng, S., He, X., Hammack, T. S., Brown, E. W., et al. (2018). Development of a novel loop-mediated isothermal amplification (LAMP) assay for the detection of *Salmonella* ser. Enteritidis egg products. *Food Control* 88, 190–197. doi: 10.1016/j.foodcont.2018.01.006

Kaneko, I., Kawana, T., Fukushima, E., and Suzutani, T. (2007). Tolerance of loop-mediated isothermal amplification to a culture medium and biological substances. *J. Biochem. Biophys. Methods* 70, 499–501. doi: 10.1016/j.jbbm.2006.08.008

Kaneko, I., Miyamoto, K., Mimura, K., Yumine, N., Utsunomiya, H., Akimoto, S., et al. (2011). Detection of enterotoxigenic Clostridium perfringens in meat samples by using molecular methods. *Appl. Environ. Microbiol.* 77, 7526–7532. doi: 10.1128/aem.06216-11

Kashir, J., and Yaqinuddin, A. (2020). Loop mediated isothermal amplification (LAMP) assays as a rapid diagnostic for COVID-19. *Med. Hypotheses* 141:109786. doi: 10.1016/j.mehy.2020.109786

Kuh, G. D. (2008). *High-Impact Educational Practices: What They Are, Who Has Access to Them, and Why They Matter*. Washington, D.C: Association of American Colleges and Universities.

Kuh, G. D., and O'Donnell, K. (2013). *Ensuring Quality & Taking High-Impact Practices to Scale with Case Studies by Sally Reed*. Washington, DC: Association of American Colleges and Universities.

Law, J. W., Ab Mutalib, N. S., Chan, K. G., and Lee, L. H. (2014). Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. *Front. Microbiol.* 5:770. doi: 10.3389/fmicb.2014.00770

Lin, Q., Xu, P., Li, J., Chen, Y., and Feng, J. (2017). Direct bacterial loop-mediated isothermal amplification detection on the pathogenic features of the nosocomial pathogen - Methicillin resistant Staphylococcus aureus strains with respiratory origins. *Microb. Pathog.* 109, 183–188. doi: 10.1016/j.micpath.2017.05.044

Martzy, R., Kolm, C., Brunner, K., Mach, R. L., Krska, R., and Šinkovec, H. (2017). A loop-mediated isothermal amplification (LAMP) assay for the rapid detection

of Enterococcus spp. in water. *Water Res.* 122, 62–69. doi: 10.1016/j.watres.2017.05.023

Mori, Y., and Notomi, T. (2009). Loop-mediated isothermal amplifi cation (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. *J. Infect. Chemother.* 15, 62–69. doi: 10.1007/s10156-009-0669-9

Smyth, D.S. (2017). An Authentic Course-Based Research Experience in Antibiotic Resistance and Microbial Genomics. *Sci. Educ. Civic Engage.* 9:59.

Tang, T., Cheng, A., Wang, M., Li, X., He, Q., Jia, R., et al. (2012). Development and clinical verification of a loop-mediated isothermal amplification method for detection of *Salmonella* species in suspect infected ducks. *Poult. Sci.* 91, 979–986. doi: 10.3382/ps.2011-01992

Wang, X. R., Wu, L. F., Wang, Y., Ma, Y. Y., Chen, F. H., and Ou, H. L. (2015). Rapid detection of Staphylococcus aureus by loop-mediated isothermal amplification. *Appl. Biochem. Biotechnol.* 175, 882–891. doi: 10.1007/s12010-014-1328-x

Warghane, A., Misra, P., Bhose, S., Biswas, K. K., Sharma, A. K., Reddy, M. K., et al. (2017). Development of a simple and rapid reverse transcription-loop mediated isothermal amplification (RT-LAMP) assay for sensitive detection of *Citrus tristeza virus*. *J. Virol. Methods* 250, 6–10. doi: 10.1016/j.jviromet.2017.09.018

Yamazaki, W., Taguchi, M., Kawai, T., Kawatsu, K., Sakata, J., Inoue, K., et al. (2009). Comparison of loop-mediated isothermal amplification assay and conventional culture methods for detection of Campylobacter jejuni and Campylobacter coli in naturally contaminated chicken meat samples. *Appl. Environ. Microbiol.* 75, 1597–1603. doi: 10.1128/aem.02004-08

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Is Community Relevance Enough? Civic and Science Identity Impact of Microbiology CUREs Focused on Community Environmental Justice

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Course-based undergraduate research experiences (CUREs) often involve a component where the outcomes of student research are broadly relevant to outside stakeholders. We wanted to see if building courses around an environmental justice issue relevant to the local community would impact students' sense of civic engagement and appreciation of the relevance of scientific research to the community. In this quasi-experimental study, we assessed civic engagement and scientific identity gains (N = 98) using pre- and post-semester surveys and open-ended interview responses in three different CUREs taught simultaneously at three different universities. All three CURES were focused on an environmental heavy metal pollution issue predominantly affecting African–Americans in Birmingham, Alabama. While we found increases in students' sense of science efficacy and identity, our team was unable to detect meaningful changes in civic engagement levels, all of which were initially quite high. However, interviews suggested that students were motivated to do well in their research because the project was of interest to outside stakeholders. Our observations suggest that rather than directly influencing students' civic engagement, the "broadly relevant" component of our CUREs engaged their pre-existing high levels of engagement to increase their engagement with the material, possibly influencing gains in science efficacy and science identity. Our observations are consistent with broader community relevance being an important component of CURE success, but do not support our initial hypothesis that CURE participation would influence students' attitudes toward the civic importance of science.

Keywords: civic engagement, community, microbiology laboratory education, science identity, course-based undergraduate research experience

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INTRODUCTION

Course-based undergraduate research experiences (or CUREs) are becoming increasingly popular in higher education in the United States for their ability to engage university students in authentic research in the context of a course (AAAS, 2009). CUREs are often set apart from more traditional laboratory experiences as allowing students to: (1) learn authentic science practices; (2) discover outcomes not already known to the instructors or students; (3) collaborate; (4) repeat and iterate

experiments; and (5) engage with a "broadly relevant" project (Auchincloss et al., 2014). Research results are mixed regarding how much each of these factors contribute to positive student learning outcomes, and not all educators agree that all five factors are necessary parts of every CURE (Ballen et al., 2018). In particular, the "broadly relevant" criterion has come under scrutiny, as it is the most difficult of the five criteria to achieve in practice, particularly at less well-funded institutions. In the meeting report of CUREnet, an online CURE repository, the authors posit "CUREs involve students in work that fits into a broader scientific endeavor that has meaning beyond the particular course context" (Auchincloss et al., 2014). Put another way, broad relevance indicates the presence of, or need for, an outside stakeholder.

Strategies for engaging stakeholders in CUREs vary. The most common solution is to couch the CURE in the context of a larger research project, essentially crowd-sourcing research using student labor (e.g., PARE, Genné-Bacon and Bascom-Slack, 2018; HHMI SEA-PHAGES, Jordan et al., 2014; Tiny Earth, Basalla et al., 2020; and Small World Initiative, Davis et al., 2017) but some effort has been placed into achieving broad relevance through more direct interactions with stakeholders. Ballen et al. (2018) suggested that something as simple as students e-mailing products of their work to an external professor stakeholder could qualify, although Corwin et al. (2018) argue an e-mail may not be sufficient to classify as a broader impact. A wide range of other approaches have also been considered, including acknowledging students in a manuscript, contributing to external databases, and partnering with specific community needs (Corwin et al., 2018). For example, Malotky et al. (2020) paired student research projects like assessing literacy with community-partner engagement such as having students read to an elderly population. These types of examples straddle the line between CUREs and Service-Learning, where course material achieves goals complimentary with direct needs within the community (Smith, 2003; Mendoza et al., 2020).

Student scientific gains in CUREs are also well-documented (Gormally et al., 2009): students i) gain confidence in their ability to do science and to perceive themselves as scientists (Hanauer et al., 2016), ii) are more likely to persist in STEM majors (Estrada et al., 2011), and iii) are more likely to pursue science as a career (Lopatto, 2007). Situated learning theory (Korthagen, 2010) posits that immersion in the cultural milieu of science in the context of authentic research can foster a student's sense of belonging to the field, but it also suggested to us that broad community relevance in a CUREs might increase feelings of involvement with the impacted stakeholder communities. Sanders and Hirsch (2014) found students who were part of a civically engaged CURE were more likely to want to go into a science career. However, the degree to which broader impacts in CUREs play a role in science identity and persistence gains remains an open question (Corwin et al., 2015, 2018).

Here, we present our results from implementing three different but simultaneous CUREs related to the same environmental justice issue in North Birmingham, Alabama. Since the late 19th century, large-scale industrial activity and political corruption have created a legacy of heavy metal and toxic organic pollution in Birmingham's 35207 zip code (Allen et al., 2019). In 2012, the evidence of contamination was so significant and widespread that it was declared the 35th Avenue Superfund Site by the Environmental Protection Agency (this site will henceforth be referred to by its zip code, 35207). The residents of 35207 are predominantly African–American, of low socioeconomic status, and suffer from elevated risk of respiratory disease relative to similar populations due to their chronic exposure to toxic heavy metals in their living environment (Allen et al., 2019). As a consequence, understanding how the legacy of pollution influences both the human and environmental health of this area is an issue of social and environmental justice that is directly relevant to members of the greater Birmingham community including all of the students in our study.

The research component of our CUREs focused on various aspects of the co-evolution of heavy metal tolerance and antibiotic resistance in bacteria. Bacteria exposed to either antibiotic or heavy metal stress often evolve resistance to both simultaneously because there is substantial overlap in the genes underlying these phenotypes (Baker-Austin et al., 2006). Thus, as shown in previous contaminated sites (Chen et al., 2019), the possibility exists that long-term exposure to elevated heavy metal concentrations has led to the development of a reservoir of antibiotic-resistant bacteria in the 35207 environment, particularly in the soil. Either through direct infection by environmental microbes, or because of horizontal transfer of resistance genes between environmental and human-associated microbes, 35207 residents could be exposed to elevated risk of recalcitrant antibiotic-resistant infections (Seiler and Berendonk, 2012), although the clinical aspects of this research project are beyond the scope of the student projects described here. Students in our CUREs assessed the degree to which soil bacteria in 35207, compared to a demographically comparable control zip code without elevated heavy metal exposure, were influenced by the history of pollution. Specific research projects are briefly described in the Methods section, and our scientific results will be published in a forthcoming manuscript.

The possible microbiological impact of pollution on human health in 35207 has clear relevance to 35207 residents, and we sought to incorporate that broad community relevance in our courses by i) assigning reading material and videos about 35207 and its residents, ii) incorporating 35207 and data associated with it into lectures, iii) tasking students to generate relevant data, and iv) assigning students to generate products (student-created videos or data) designed to be shared with community stakeholders after the class was completed. These broadly relevant tasks (readings, working with real data, and creating videos) are straightforward educational practices which do not rely on an outside stakeholder during the course and thus could be ideal for CURE educators wishing to incorporate broad relevance in their course. We hypothesized that the inclusion of broader social relevance into the coursework would lead to increases in students' senses of civic engagement and appreciation for the importance of scientific research for achieving local community goals, while also achieving the increases in scientific efficacy and self-identification commonly observed in CUREs. Specifically, we sought to answer the following questions:

1. To what degree do students at these three universities already agree with statements about civic engagement, science identity, and the role of science in the community prior to their participation in their CURE research project?
2. Are our attempts to include broader community relevance sufficient for students to make gains in their sense of community engagement, and does community engagement interact with gains in science identity and/or science efficacy?

MATERIALS AND METHODS

Preparation

Researchers (SA and QR) collected six homogenized soil samples from public areas in 35207 and six homogenized soil samples from the neighboring control 35214 zip code. 35214 has similar demographics and physical characteristics, but no history of heavy metal pollution. Soil was tested for heavy metal contamination by Sutherland Environmental Testing, which found elevated concentrations of lead, manganese, and zinc in the 35207 zip code areas (data not shown). Approximately 1 g of soil from each public area was given to the teaching teams at the three universities for their analyses; sample IDs were randomized before distribution so that neither students nor instructors would know where a given sample came from.

Course Descriptions

Participants were recruited during a 14-week Fall 2019 semester from classes at three universities in Birmingham, Alabama. Across all classes, we first identified the CURE learning objectives and research goals based on the recommendations in the CURE literature (Shortlidge and Brownell, 2016; Cooper et al., 2017), which are summarized in **Table 1**.

University 1 (U1), the University of Alabama at Birmingham, is a research-intensive public university and doctorate granting institution, Microbial Ecology enrolled 15 students (including both graduate and undergraduate students) for 50-min evening classes Monday, Wednesday, and Friday. Microbial Ecology is cross-listed as an upper division (400-level) undergraduate course as well as a graduate course, with a prerequisite of introductory microbiology. The course was a mixture of lecture, usually on Monday and Wednesday, and bioinformatics workshops, usually on Friday. The lecture portion examined microbial life in natural habitats, such as soil and aquatic ecosystems, with a focus on taxonomic diversity and ecosystem-scale biogeochemical processes. During the workshops, students learned the Standard Operating Procedure for processing next-generation DNA sequence data using mothur (Kozich et al., 2013). Total genomic DNA was extracted from all 12 soil samples with Qiagen PowerSoil kits and used to generate Illumina MiSeq 16S rRNA barcode libraries (Caporaso et al., 2012). In the last month of the semester, U1 students worked in 3-person teams to execute their own bioinformatics projects testing 35207-related hypotheses with this data.

University 2 (U2), Birmingham Southern College, is a private, undergraduate-only liberal arts college. The Cell and Molecular Biology course had an enrollment of 120 undergraduate students. They all attended the same lecture at the same time, however, they were split into six hour-long laboratory sections on Monday and Wednesday mornings. The course served as a 100-level prerequisite for all upper-level biology courses. The teaching team previously pioneered the Metal and Antibiotic Resistance Evolution (MARE) Project, which had students explore the effects of long-term industrial pollution in Birmingham using environmental samples from a Birmingham landmark, Sloss Furnace. Aside from where the environmental samples were collected (North Birmingham residential areas instead of Sloss Furnace), there were no substantial changes to how this course had previously been taught. In their labs, after learning aseptic technique and other fundamental skills (e.g., Gram staining and PCR), students used culture-based methods to screen 35207 and 35214 bacteria for resistance to antibiotics and heavy metals. Agar media had either no addition or contained antibiotics (ampicillin, tetracycline, ciprofloxacin, or a carbapenem drug) or heavy metals (Zn, Mn, and Pb) at concentrations that were previously established to inhibit microbial growth.

University 3 (U3), Samford University, is a private Christian university that awards both undergraduate and graduate degrees. At U3, 48 students across 2 morning sections (24 students per section) took the Foundations of Biology lecture and lab class, which served as a 200-level prerequisite for all upper-level biology courses. The class, composed mostly of freshmen pre-health students and biology majors, met MWF for 110 min per session. The content covered basic biochemistry, cellular metabolism, molecular biology and genetics, taxonomy, and phylogeny, as well as evolution. In the laboratory portion of the course, students began by learning basic microbiology and molecular biology skills. About mid-semester, students worked in 4-person teams to quantify the abundance of total bacteria as well as antibiotic and heavy metal resistance genes in soil-extracted DNA using quantitative PCR (Di Cesare et al., 2016).

In addition to completing the research projects described in **Table 1**, students in all courses were required to read a news article on the environmental pollution in North Birmingham (Pillion, 2017). Instructors also regularly discussed the importance of the study with students (instructional tools available upon request). Additionally, because student learning objectives in all courses included collaboration and communication, all instructors agreed to include a collaborative video assignment as a part of the course. The video assignments at U1, U2, and U3 were worth 10, 5, and 7.5%, percent of student final grades, respectively. In these assignments, students were tasked with creating 3–5 min instructional videos to share with their student colleagues at the other universities what they had accomplished through their research. These videos needed to also explain the civic engagement component where students contextualized how their specific research aim was related to the environmental justice problem in North Birmingham. Instructors had meetings in-person or through video chat twice a month to provide updates related to education research or

TABLE 1 | Curricular research goals and learning objectives among three non-synchronous CURE courses.

Course	Research goals	Student goals
Microbial Ecology (U1)	1. Discover bacterial taxa that are shared between metal-exposed people and their environments that are absent (or rare) in metal-naive populations 2. Analyze differences in metabolic capacities of metal-exposed soils in comparison with metal-naive soils	1. Master experimental design/scientific method 2. Gain confidence with command-line bioinformatics tools and their interpretation 3. Learn to apply ecological principles to human health questions 4. Collaborate with other student researchers to accomplish a larger task with social relevance
Cell and Molecular Biology (U2)	1. Determine prevalence of metal and antibiotic resistant bacteria from contaminated soil 2. Identify resistant bacteria using morphological, biochemical, and molecular data	1. Apply scientific method to look at the relationship between metal and antibiotic resistance 2. Discuss role of science in social discourse 3. Communicate scientific results
Foundations of Biology (U3)	1. Isolate bacteria from soil environments 2. ID/quantify genes that confer antibiotic/heavy metal resistance in tainted soils	1. Apply modern scientific method to a practical experimental design/interpretation of data 2. Interact with peer group to communicate results

curricular progress, including supplies needed for students to carry out their research project.

Recruitment and Procedure

We surveyed students enrolled in all three courses prior to their first assignment related to experimental design (**Table 2**) using an online Google survey (see **Supplementary Material**). We also asked students to self-report their professional goals from a list of academic and non-academic careers at the end of the survey (see **Supplementary Material**). Students were asked to complete the surveys, outside of class time, 1 week prior to beginning their CURE projects, though students at U1 were given 20 min at the beginning of a class period to incentivize their participation. Students completed the same survey as a post survey in the last week of class. Students at all three universities identified themselves using their student ID numbers to enable pre and post data to be matched as well as to associate survey responses with demographic information (including race, gender, class standing, ACT or SAT equivalent and cumulative GPA) from the university's registrar. Additionally, a researcher other than the professor assigning grades (either SA or RA) came into each course in the last week of the semester, prior to final exams, to gage students' attitudes through recorded class discussions using semi-structured interviews. To accommodate the professor and course scheduling, U2 and U3 interviews took place following course-wide review sessions for the final exam and the interviews at U1 took place immediately prior to students taking their final examination. Note that there were two separate course section interviews for U2 but these student comments were combined for purposes of analysis as the instructional curriculum was identical save for the course meeting times. In sum, at U1, eight consenting students took the pre-survey (53% of the class) and two students completed the post surveys (13% of the class). Six U1 students participated in the end of semester interviews. At U2, there were 92 students who consented (77% of the class) and took the pre-surveys and 38 who took the post surveys (32% of the class). 9 U2 students participated in the interviews. In the U3 course, nine students consented (19% of the class) and completed the pre-surveys and five students completed the post surveys (11% of the class). There were 36 total U3 students who were interviewed during two class-wide interviews, one interview with 17 students present and the other with 19 students present. Class discussions were manually transcribed and identifying information was removed.

TABLE 2 | Procedures across all three CUREs.

Procedure	Length of time required of participants	Total # of times procedure performed
Google surveys (pre and post surveys)	20 min × 2 = 40 min	2 (Pre semester and Post semester)
Discussion interviews	20–50 min	1 (Post semester)

Instruments

We compiled questions from three Likert-scaled surveys (**Box 1**) relevant to our learning goals, where lower Likert numbers represent greater levels of agreement with the question. To survey engagement with the community, we used the Civic Engagement Scale (CES), a validated survey for gaging student attitudes and behaviors of engagement related to scholastic service-learning (Doolittle and Faul, 2013). We included CES questions on community mindedness (questions 1, 2, and 6), but excluded questions on volunteering as volunteering was not a focus of any of our courses. To test civic responsibility, we chose the Civic Attitudes about the Relevance of Science (CARS) survey (Siegel and Ranney, 2003) in order to question students' perceptions of how science shapes attitudes on i) the environment (questions 5 and 42), ii) waste from factories, a specific focus of our courses (question 28), and iii) using evidence to make decisions (question 38). We used questions from the Persistence In The Sciences (PITS) Instrument (Hanauer et al., 2016) to assess Project Ownership Content and Emotion (PITS subsection question 1, abbreviated henceforth as "POCE"); Self-Efficacy (PITS subsection questions 1–5, abbreviated as "SE"), Science Identity (PITS subsection questions 1–4 abbreviated as "SI") and Scientific Community Values (PITS subsection questions 3 and 4, abbreviated as "SCV"). To account for students from different populations and backgrounds possibly having different ideas about the word community, we chose to ask them to define "community" and "community of scientists" prior to their first use in our survey.

BOX 1 | Survey questions and related acronyms. Full question format is available in **Supplementary Material**.

Civic Engagement Survey and Attitudes about the Relevance of Science

CES1. I feel responsible for my community.
CES2. I believe I should make a difference in my community.
CES6. I believe that it is important to be informed of community issues.
CARS5. Learning science helps me understand about the environment.
CARS28. Science has nothing to do with local issues, such as waste from nearby factories.
CARS38. Collecting evidence is an important part of making a decision.
CARS42. Knowledge of science will help me protect the environment.

Persistence in the Sciences (PITS)

PITS POCE1. My research will help to solve a problem in the world.
PITS SE1. I am confident that I can use technical science skills (use of tools, instruments, and techniques).
PITS SE2. I am confident that I can generate a research question to answer. (POC3)
PITS SE3. I am confident that I can figure out what data/observations to collect and how to collect them.
PITS SE4. I am confident that I can create explanations for the results of the study.
PITS SE5. I am confident that I can use scientific literature and reports to guide my research.
PITS SI1. I have a strong sense of belonging to the community of scientists.
PITS SI2. I derive great personal satisfaction from working on a team that is doing important research.
PITS SI3. I have come to think of myself as a 'scientist'.
PITS SI4. I feel like I belong in the field of science.
PITS SCV3. I am a person who thinks that scientific research can solve many of today's world challenges.
PITS SCV4. I am a person who feels discovering something new in the sciences is thrilling.

Qualitative Procedure and Analysis

In our open interviews with students, we asked them i) the purpose of the course as they understood it, ii) what they learned and their motivations, iii) how the course influenced their career direction, and iv) how their views of science changed. We specifically chose not to prompt students about experimental design, civic engagement, or civic responsibility in order to avoid priming their responses, thus encouraging authentic discussions related to those topics. We used content analysis (Hsieh and Shannon, 2005) to apply the same themes present in the semi-structured interview questions (see **Supplementary Material**) to categorize the responses, and these overall summaries were decided and agreed upon unanimously by the two coders (RA and SA). Lastly, we used content analysis (Hsieh and Shannon, 2005) to find themes which emerged from student definitions of "community" and came to a complete consensus for coding the students' definitions.

Statistical Analysis

Changes in student responses between the pre and post surveys were assessed using linear mixed effects models (deployed in the *lme4* package in R), with university and pre vs post as fixed effects and student ID as a random effect. We note that the incorporation of university as a fixed effect prevents the large difference in sample sizes between universities from skewing model results. Significance of terms was determined by contrasting the full model against nested subset models dropping one of the fixed effects, or the interaction between fixed effects, using the *anova* command in R. Coefficient values reported here are derived from extended marginal means estimates (using the *emmeans* package in R) applied to refined models that eliminate all non-significant terms. All other statistical tests were conducted as linear models in R; as with the linear mixed effects models, the coefficients reported came from extended marginal means estimates from refined models that omit any non-significant predictors.

IRB Oversight

This study was approved by the IRB Protocols IRB-300004139 (U1), IRB Protocol # 2017-02-011(U2), and IRB Protocol SU-EXMT-A-19-F-2 (U3).

RESULTS

Quantitative Data

Differences Between Universities

Because of the large differences between the universities and the level of instruction in the different courses, we expected that students would have significantly different baseline attitudes and skills related to our surveys. U1 students reported higher agreement to the CARS statement "Learning science helps me understand about the environment" than U3 students, and were more likely to agree with the PITS statements "I have come to think of myself as a 'scientist'" and the CARS statement "Knowledge of science will help me protect the environment" than students at either U2 or U3 (**Figure 1**). Collectively, these initial differences suggest that the more advanced students of U1 held more professional-like attitudes toward science and the value of science than did the beginning students at U2 and U3. There were no significant between-university differences in any of the other survey questions (data not shown).

We also found that gender significantly influenced student responses in different ways between the three universities we studied. Female students at U1 were more likely than female students at the other universities to agree with belonging to a community of scientists (PITS SI-1), and female students at both U1 and U2 were more likely than female students at U3 to agree with thinking of themselves like scientists (PITS SI-3). On the other hand, U3 males were significantly less likely than males at the other universities to agree with science informing knowledge of the environment (CARS5), the importance of evidence in decision planning (CARS38), and science knowledge informing environmental protection (CARS42). Male students at U3 were also less likely than U3 females to agree with these statements about making a difference in their community (CES2), needing to be informed about community issues (CES6), having satisfaction while working on a science team (PITS SI-2), feeling belonging in science (PITS SI-4), and feeling as though new things in science are thrilling (PITS SCV-4). Further, U3 male students were significantly more likely to agree with science having nothing to do with local issues (CARS Q28) than U3 female or male students from either of the other universities. See **Box 1** for statement details. Overall, U3 male students appeared

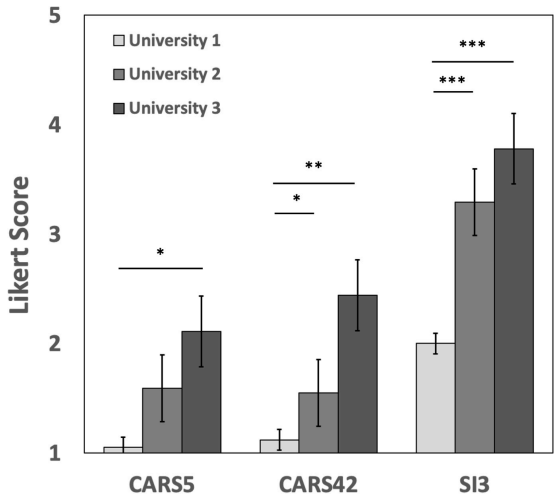

FIGURE 1 | Baseline differences in student attitudes at the three universities. There were only differences in three survey question responses between the universities. Likert scale value 5 indicates strong disagreement and value 1 strong agreement. Error bars in both panels are the 95% confidence intervals from linear model estimates. Asterisks indicate significance levels: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

to be strong outliers relative to the rest of the study group; we note however that this was a quite small sample set, consisting of only five male student entries. Importantly, gender did not influence the changes in pre to post for either males or females at any university; the differences described here appear to reflect differences in baseline attitudes and not the effects of the research curriculum on those attitudes.

Gains in Civic Engagement and Scientific Identity

We hypothesized that student agreement to statements on civic engagement would change after a semester of a course focusing on issues of local environmental justice. There was no statistical interaction between university and pre-post change in Likert responses, so the following results represent the entire student population from U1, U2, and U3. Contrary to our expectation, agreement to most of the questions from the CES and CARS instruments did not significantly change over the course of the semester (**Figure 2**). Only one CARS statement, "Science has *nothing* to do with local issues, such as waste from nearby factories," changed, but in the opposite direction from our expectation. We were surprised that the average student in the study, after taking a semester of a research course exploring the effects of factory waste, was less likely to agree that factory waste is relevant to science (**Figure 2**, question CARS28). On the pre-survey, only 2% of students responded in agreement with this statement, whereas on the post survey more than 13% of students agreed. While it is impossible to be certain, we suspect that the reverse wording of this question (i.e., "Science has *nothing* to do with local issues") may have led to confusion during the post survey; the lack of movement in the "wrong" direction on other questions, and the absence of interview responses supporting such an attitude, provides support for this possibility.

The proportion of students in agreement with the remaining CES and CARS questions was very high on both the pre and post surveys. Note that we asked students to define "community" before taking the CES portion of the survey, as ideas about community could differ across student cultures and backgrounds, but themes present in student definitions were not significant predictors of their pre or post responses on relevant questions about community (CES 1, CES 2, CES 6, or PITS-SI 1).

In contrast to our civic engagement questions, we found a significant shift toward agreement on several PITS statements: "I am confident that I can generate a research question to answer"; "I am confident that I can figure out what data/observations to collect and how to collect them"; "I am confident that I can use scientific literature and reports to guide my research"; "I have come to think of myself as a 'scientist'"; and "I am a person who thinks that scientific research can solve many of today's world challenges" (**Figure 2**). These statements include at least one representative from each of the three PITS categories we included: science efficacy, science identity, and scientific community values. As there were no statistically significant differences in the magnitude of change between universities, we conclude that CURE students at all three universities experienced significant gains in the professional virtues measured by PITS, but not in their sense of civic engagement or community responsibility.

Qualitative Data

We conducted semi-structured interviews with student participants representing each university (U1 $n = 6$, U2 $n = 9$, and U3 $n = 36$) (**Table 3**). Unsurprisingly, students' reported understanding of the purposes of their courses and their knowledge gained aligned with the respective course goals (**Table 1**). Students also consistently brought up the "real world" components of the course as impacting their engagement with the course material. For example, students in the freshmen courses (U2 and U3) but not in the graduate course (U1) explicitly noted their motivation in their research was due to local stakeholders. Students at U1 and U3, but not U2, mentioned being motivated in their research because of the real-world data and student research videos. Example quotes regarding student motivations from U1, U2, and U3 are as follows:

> *I think working with real data was a really big part of motivation for this class and I think most of the grad students feel the same way* – U1 student.

> *I think it being around the corner from where we are, kinda made us want to know what's going on. Especially when you heard soil contamination, like what is that exactly? It being so close to you makes you want to dive in and figure out what was going on* – U2 student.

> *It made me want to make sure I did it right, like, in chem lab sometimes if you don't have your data right, you can get numbers from other people if you miss something. But, with this, because it doesn't matter really, but with this, it's like you're actually making an impact on the people who are living in that area and so it made you want to like, do it well and not just do it* – U3 student.

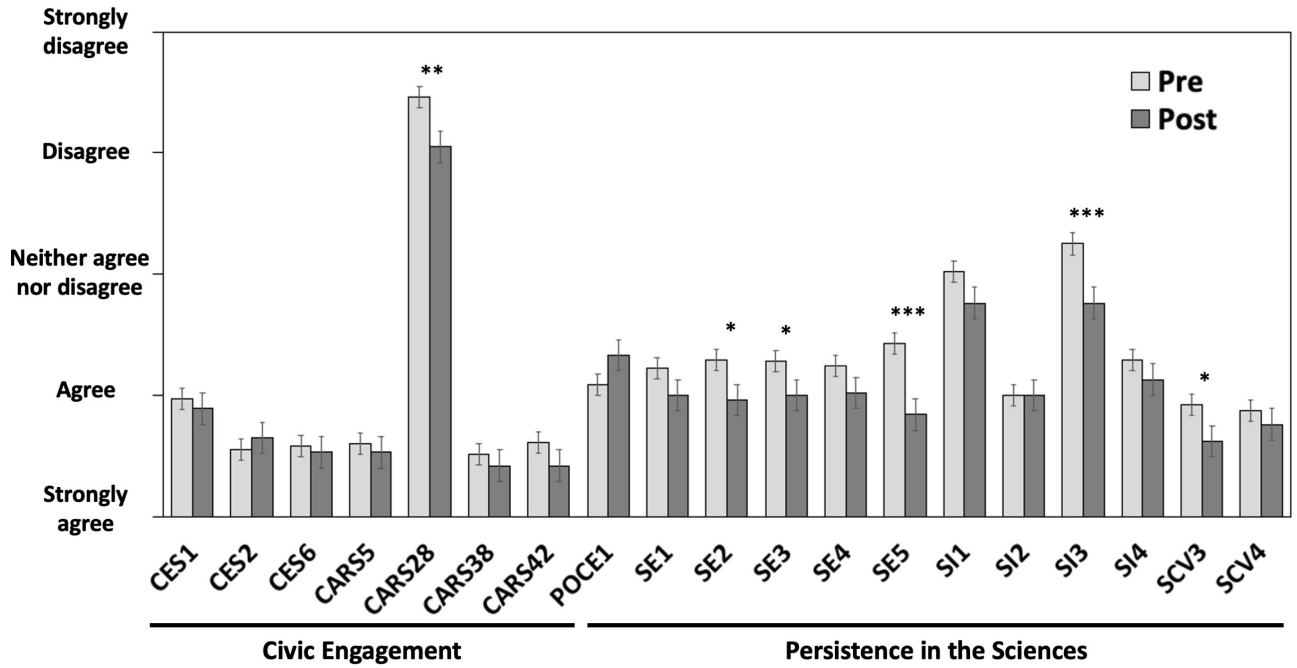

FIGURE 2 | Pre and post responses from 98 students across three simultaneous CUREs. Full questions are provided in **Box 1**. The Likert scale value 5 indicates strong disagreement and value 1 indicates strong agreement for all questions. Note CARS 28 used an inverted statement. Error bars indicate 95% confidence intervals and asterisks indicate the level of significance from extended marginal means contrasts between pre and post responses derived from linear mixed effects models. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. There was no significant difference between universities in the change between pre and post responses.

TABLE 3 | Summary of students' end-of-semester interview responses to question prompts across three non-synchronous CURE courses.

	Course		
Question prompt	**Microbial Ecology (U1)**	**Cell and Molecular Biology (U2)**	**Foundations of Biology (U3)**
Purpose	Learn microbial ecology and interactions; Superfund CURE	Heavy metal contamination of soil in Alabama	To engage in real research
Knowledge learned	Diversity of microbial ecology; Bioinformatics pipelines	Effects of heavy metal contamination at the EPA Superfund Site	Molecular biology laboratory techniques
Motivation	Real research and different projects; Increased understanding of Bioinformatics; Research videos seen by other students	Local stakeholders	Real research with unknown solutions with community stakeholders; Research videos seen by other students
Career goals	Affirmed	No changes mentioned	Affirmed or challenged
View of science	Changed; Science depends on many parts and collaboration; Research videos helped explain science projects	No changes mentioned	Changed; No predictive "right" answer; Science does not have to contradict faith

The last two question prompts, about career goals and views of science, were not addressed by students at U2 potentially because the environment in which they were interviewed – right after a study review session – may not have been conducive to a complete and nuanced discourse (RA personal observation). Career goals and views of science shifted for students at U1 and U3. U3 students explicitly mentioned that the student research videos impacted their views of science. In the **Supplementary Material**, we provide summaries of student responses to each primary question as well as our interview script.

DISCUSSION

Our primary goal in the work presented here was to investigate whether CURE courses connected to a project with environmental justice implications for the local community would influence student attitudes toward the relationship of science and scientists with the general population and the common good. Secondarily, we wanted to understand how student perceptions of the "broad relevance" of their course research – one of the commonly understood cornerstones of CURE classes – influenced their personal identification

as scientists. As we described in greater detail below, our overall conclusion was that our students already had a strong appreciation for the importance of scientific research for society and the community, and this was not changed by our courses. Despite a lack of movement in terms of civic engagement, our students nevertheless became more confident of themselves as scientists, and some of their responses during interviews suggested that the "broad relevance" of their project may have contributed to that improvement.

Civic Engagement Gains From Surveys and Interviews

Our survey data demonstrated that our students began the course with relatively high levels of community engagement. Pre-scores for CES and CARS showed considerably higher levels of agreement than those for any of our scientific identity metrics (**Figure 2**). For example, most students across all of the courses either strongly agreed or agreed that they felt responsible for their community, wanted to make a difference in their community, and needed to be informed of community issues. Civic-engagement pedagogy has permeated through education for decades (Putnam, 2000), so it is possible that previous education has played some role in these attitudes. Initially high levels of agreement limited our ability to assess student attitudinal changes in civic engagement using these particular CES and CARS instruments.

Brammer and Morton (2014) were able to tease out civic engagement themes like collaboration, effort, passion, and responsibility from open-ended student reflections in an immersive community-based semester-long public advocacy course. Likewise, our qualitative data provides us a richer story than does the CES and CARS survey results. We expected students in interviews to describe being motivated by the civic engagement parts of the course, including the students' research being useful for outside stakeholders and creating summary videos about how their research connects to the environmental justice problem. Indeed, students from all three courses reported motivations related to the broader impacts of the CURE (**Table 3**). Interestingly, we found a marked difference between how the broader impact of the course affected students depending on their course context. Students in the freshmen courses, U2 and U3, emphasized the people and geography of the local stakeholders in their motivations to take their research in the course seriously, whereas the U3 students unanimously expressed that sharing research videos with other scientists was a motivating factor for their continued work on the research projects (**Table 3**). U1 graduate students were more likely to focus on the scientific impacts of their research as a motivating factor, while still acknowledging the importance of the environmental justice component of the work.

Overall, we found that the broad relevance of projects involving environmental justice and the local community had an effect on student motivation in the course, even though the students had no direct interactions with actual stakeholders in the 35207 community or with other professional scientists working on 35207-related projects. Creating avenues of communication, not just with other student researchers, but with these communities at large, e.g., through Service-Learning pedagogies, could be fruitful improvements for future incarnations of these or similar courses (Smith, 2003).

Science Identity Gains From Surveys and Interviews

Agreement with several of the statements related to science identity, science efficacy, and scientific values increased from the beginning to the end of the semester (**Figure 2**). Students across all three universities left their CURE more confident in generating a research question, collecting data, using scientific literature, thinking of themselves as scientists, and thinking research could solve challenges in the world, which are similar to gains reported elsewhere in the literature for CUREs (Gormally et al., 2009; Estrada et al., 2011, Hanauer et al., 2016; Adkins et al., 2018). Many of these results were also corroborated by the interview data (**Table 3**). Unsurprisingly, all interview responses about the purpose of the course aligned with research goals or learning objectives and corresponded closely with the curricula for each class (**Table 1**). The fact that similar gains were experienced across very different types of classes, and against very different starting levels of scientific identity and experience, provides further support for the impact of the CURE format on developing scientific confidence in diverse students.

Previous research suggested that civically engaged CUREs might improve scientific persistence (Sanders and Hirsch, 2014), and indeed, this course affirmed career trajectories for several U1 students (**Table 3**), and some U2 students explained how these new understandings made them want to explore science as an occupation while at least one student explained how the course made them realize scientific research was not for them (**Table 3**). One benefit of CUREs is that they allow students to have authentic experiences so as to ground these important decisions before entering the job market (Lopatto, 2007). More specifically, U3 students told interviewers they now saw science as a field that is much more open ended than they had understood previously; shifts in science attitudes like this are common outcomes of CUREs (Olimpo et al., 2016).

Finally, we hypothesized that including a conspicuous community relevance component to students' research would strengthen the "broader relevance" pillar of our CURE, and may therefore have contributed to greater gains in scientific confidence than courses with more abstract or process-oriented research goals. As described above, our hypothesis that our environmental justice focus would increase students' sense of civic engagement was rejected. However, our interviews clearly showed that students were motivated by the real-world implications of their work, and it is possible that their already high levels of civic engagement underpin that motivation. Thus, instead of our CURE increasing their civic engagement, our students' civic engagement may have increased the efficacy of the CURE. Our study cannot directly test this possibility, but we suggest that future work should explicitly measure the impact on scientific identity and confidence of increasingly direct interactions with outside stakeholders in CUREs, ranging

from the purely procedural (e.g., submitting data to a national database) to the explicitly personal (e.g., Service-Learning).

Limitations and Future Directions

Our ability to analyze some of our research questions was limited by the fact that not all students completed the entire questionnaire, and in general we had disappointing levels of participation in at least one of our classes. In general, we suspect that the time investment required to complete the entire survey, which included experimental design questions not reported in this manuscript, may have contributed to the relatively low student participation, particularly on the post survey. Some demographic information was also not available for all students, limiting the statistical power of some of our demographic analyses. The size of the student cohorts at the three universities were also quite different, which has the potential to skew aggregate results in favor of the larger U2 cohort, although we attempted to control for this possibility using a linear mixed effects statistical procedure. Moreover, we only surveyed one semester's worth of classes, and our results may only be representative of students taking these classes in a Fall semester. We acknowledge that this study represents an anecdote in only one city in one country and in one semester, and so more work should be done investigating how broad relevance affects the success of CUREs for a broader demographic of students.

While not including local stakeholders was both situational and intentional, we know this may have limited the ability of the students to appreciate the reciprocal relationship between their scientific work and its human impacts. We are hopeful that we can make this direct interaction a reality in future courses. In particular, we recommend that future attempts to link CUREs to community-relevant projects involve a Service-Learning component. This can enable students to build relationships with local stakeholders, thereby working to have students see science as more than an intellectual pursuit, but also a pursuit that is inextricably linked with human lives.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Boards of UAB, Birmingham Southern College, and Samford University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BB and JM taught courses analyzed in the study. SA, BB, and JM designed the study and IRB protocol. SA and QR assisted in curricula preparation. BB and JM consented students and invited students to participate. SA and RA interviewed students, transcribed interviews, and coded interviews. JM analyzed the Likert data, interpreted the analysis, and created figures. SA, BB, and JM wrote the manuscript with feedback from RA and QR. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.578520/full#supplementary-material

REFERENCES

AAAS (2009). *Vision and Change A Call to Action*. Washington, DC: AAAS.

Adkins, S. J., Rock, R. K., and Morris, J. J. (2018). Interdisciplinary STEM education reform: dishing out art in a microbiology laboratory. *FEMS Microbiol. Lett.* 365:fnx245.

Allen, S., Fanucchi, M. V., McCormick, L. C., and Zierold, K. M. (2019). The search for environmental justice: the story of North birmingham. *Int. J. Environ. Res. Public Health* 16:2117. doi: 10.3390/ijerph16122117

Auchincloss, L. C., Laursen, S. L., Branchaw, J. L., Eagan, K., Graham, M., Hanauer, D. I., et al. (2014). Assessment of course-based undergraduate research experiences: a meeting report. *CBE Life Sci. Educ.* 13, 29–40. doi: 10.1187/cbe.14-01-0004

Baker-Austin, C., Wright, M. S., Stepanauskas, R., and McArthur, J. V. (2006). Co-selection. of antibiotic and metal resistance. *Trends Microbiol.* 14, 176–182 doi: 10.1016/j.tim.2006.02.006

Ballen, C. J., Thompson, S. K., Blum, J. E., Newstrom, N. P., and Cotner, S. (2018). Discovery and broad relevance may be insignificant components of course-based undergraduate research experiences (CUREs) for non-biology majors. *J. Microbiol. Biol. Educ.* 19, 19–63.

Basalla, J., Harris, R., Burgess, E., Zeedyk, N., and Wildschutte, H. (2020). Expanding the Small World Initiative and Tiny Earth to genomics: a bioinformatics approach for an undergraduate class to characterize antagonistic strains. *FEMS Microbiology Letters* 367:fnaa018. doi: 10.1093/femsle/fnaa018

Brammer, L. R., and Morton, A. (2014). Course-based civic engagement: understanding student perspectives and outcomes. *Int. J. Scholarship Teach. Learn.* 8:e080109.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., et al. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6, 1621–1624. doi: 10.1038/ismej.2012.8

Chen, J., Li, J., Zhang, H., Shi, W., and Liu, Y. (2019). Bacterial heavy-metal and antibiotic resistance genes in a copper Tailing Dam Area in Northern China. *Front. Microbiol.* 10:1916. doi: 10.3389/fmicb.2019.01916

Cooper, K. M., Soneral, P. A., and Brownell, S. E. (2017). Define your goals before you design a CURE: a call to use backward design in planning course-based undergraduate research experiences. *J. Microbiol. Biol. Educ.* 18:18.2.30.

Corwin, L. A., Dolan, E. L., Graham, M. J., Hanauer, D. I., and Pelaez, N. (2018). The need to be sure about CUREs: discovery and relevance as critical elements of CUREs for nonmajors. *J. Microbiol. Biol. Educ.* 19:1683.

Corwin, L. A., Graham, M. J., and Dolan, E. L. (2015). Modeling course-based undergraduate research experiences: an agenda for future research and evaluation. *CBE Life Sci. Educ.* 14:es1. doi: 10.1187/cbe.14-10-0167

Davis, E., Sloan, T., Aurelius, K., Barbour, A., Bodey, E., Clark, B., et al. (2017). Antibiotic discovery throughout the small world initiative: a molecular strategy to identify biosynthetic gene clusters involved in antagonistic activity. *MicrobiologyOpen* 6:e00435. doi: 10.1002/mbo3.435

Di Cesare, A., Eckert, E. M., D'Urso, S., Bertoni, R., Gillan, D. C., Wattiez, R., et al. (2016). Co-occurrence of integrase 1, antibiotic and heavy metal resistance genes in municipal wastewater treatment plants. *Water Res.* 94, 208–214. doi: 10.1016/j.watres.2016.02.049

Doolittle, A., and Faul, A. C. (2013). Civic engagement scale: a validation study. *Sage Open* 3:2158244013495542.

Estrada, M., Woodcock, A., Hernandez, P. R., and Schultz, P. W. (2011). Toward a model of social influence that explains minority student integration into the scientific community. *J. Educ. Psychol.* 103:206. doi: 10.1037/a0020743

Genné-Bacon, E. A., and Bascom-Slack, C. A. (2018). The PARE project: a short course-based research project for national surveillance of antibiotic-resistant microbes in environmental samples. *J. Microbiol. Biol. Educ.* 19:19.3.97.

Gormally, C., Brickman, P., Hallar, B., and Armstrong, N. (2009). Effects of inquiry-based learning on students' science literacy skills and confidence. *Int. J. Scholarsh. Teach. Learn.* 3:n2.

Hanauer, D. I., Graham, M. J., and Hatfull, G. F. (2016). A measure of college student persistence in the sciences (PITS). *CBE Life Sci. Educ.* 15:ar54. doi: 10.1187/cbe.15-09-0185

Hsieh, H. F., and Shannon, S. E. (2005). Three approaches to qualitative content analysis. *Qual. Health Res.* 15, 1277–1288. doi: 10.1177/1049732305276687

Jordan, T. C., Burnett, S. H., Carson, S., Caruso, S. M., Clase, K., DeJong, R. J., et al. (2014). A broadly implementable research course in phage discovery and genomics for first-year undergraduate students. *mBio* 5:e001051-13. doi: 10.1128/mBio.01051-13

Korthagen, F. A. (2010). Situated learning theory and the pedagogy of teacher education: towards an integrative view of teacher behavior and teacher learning. *Teach. Teach. Educ.* 26, 98–106. doi: 10.1016/j.tate.2009.05.001

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., and Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ. Microbiol.* 79, 5112–5120. doi: 10.1128/aem.01043-13

Lopatto, D. (2007). Undergraduate research experiences support science career decisions and active learning. *CBE Life Sci. Educ.* 6, 297–306. doi: 10.1187/cbe.07-06-0039

Malotky, M. K., Mayes, K. M., Price, K. M., Smith, G., Mann, S. N., Guinyard, M. W., et al. (2020). Fostering inclusion through an interinstitutional, community-engaged, course-based undergraduate research experience. *J. Microbiol. Biol. Educ.* 21:21.1.31. doi: 10.1128/jmbe.v21i1.1939

Mendoza, D. A., Adkins, S. J., Bhatt, J., Morris, J. J., and Raut, S. A. (2020). Service-learning curriculum increases climate change awareness. *Sci. Educ. Civic Engag.* 12:341829492.

Olimpo, J. T., Fisher, G. R., and DeChenne-Peters, S. E. (2016). Development and evaluation of the Tigriopus course-based undergraduate research experience: impacts on students' content knowledge, attitudes, and motivation in a majors introductory biology course. *CBE Life Sci. Educ.* 15:ar72. doi: 10.1187/cbe.15-11-0228

Pillion, D. (2017). *North Birmingham's 35th Ave EPA Superfund Site Explained*. Available online at: https://www.al.com/news/birmingham/2017/05/north_birmingham_35th_ave_superfund.html (accessed April 1, 2020).

Putnam, R. (2000). *Bowling Alone: The Collapse and revival of American Community*. New York, NY: Simon and Schuster. doi: 10.1145/358916.361990

Sanders, E. R., and Hirsch, A. M. (2014). Immersing undergraduate students into research on the metagenomics of the plant rhizosphere: a pedagogical strategy to engage civic-mindedness and retain undergraduates in STEM. *Front. Plant Sci.* 5:157. doi: 10.3389/fpls.2014.00157

Seiler, C., and Berendonk, T. U. (2012). Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Front. Microbiol.* 3:399. doi: 10.3389/fmicb.2012.00399

Shortlidge, E. E., and Brownell, S. E. (2016). How to assess your CURE: a practical guide for instructors of course-based undergraduate research experiences. *J. Microbiol. Biol. Educ.* 17:399. doi: 10.1128/jmbe.v17i3.1103

Siegel, M. A., and Ranney, M. A. (2003). Developing the changes in attitude about the relevance of science (CARS) questionnaire and assessing two high school science classes. *J. Res. Sci. Teach. Off. J. Natl. Assoc. Res. Sci. Teach.* 40, 757–775. doi: 10.1002/tea.10110

Smith, L. F. (2003). Why clinical programs should embrace civic engagement, service learning and community based research. *Clin. L. Rev.* 10, 723.

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Analysis of Microbial Water Contamination, Soil Microbial Community Structure, and Soil Respiration in a Collaborative First-Year Students as Scholars Program (SAS)

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The persistence of college students in STEM majors after their first-year of college is approximately 50%, with underrepresented populations displaying even higher rates of departure. For many undergraduates, their first-year in college is defined by large class sizes, poor access to research faculty, and minimal standing in communities of scholars. Pepperdine University and Whittier College, funded by a National Science Foundation award to Improve Undergraduate Stem Education (NSF IUSE), partnered in the development of first-year classes specifically geared to improve student persistence in STEM and academic success. This Students as Scholars Program (SAS) engaged first-year undergraduates in scholarly efforts during their first semester in college with a careful approach to original research design and mentoring by both faculty and upperclassmen experienced in research. Courses began by introducing hypothesis formulation and experimental design partnered with the scientific focus of each course (ecological, biochemical, microbiological). Students split into research teams, explored the primary literature, designed research projects, and executed experiments over a 6–7 week period, collecting, analyzing, and interpreting data. Microbiology-specific projects included partnerships with local park managers to assess water quality and microbial coliform contamination at specified locations in a coastal watershed. In addition, students explored the impact of soil salinity on microbial community structure. Analysis of these samples included next-generation sequencing and microbiome compositional analysis via collaboration with students from an upper division microbiology course. This cross-course collaboration facilitated additional student mentoring opportunities between upperclassmen and first-year students. This approach provided first-year students an introduction to the analysis of complex data sets using bioinformatics and statistically reliable gas-exchange replicates. Assessment of the impact of this program revealed students to view the research as challenging, but confidence building as they

take their first steps as biology majors. In addition, the direct mentorship of first-year students by upperclassmen and faculty was viewed positively by students. Ongoing assessments have revealed SAS participants to display a 15% increased persistence rate in STEM fields when compared to non-SAS biology majors.

Keywords: first-year seminar, microbiome, plant physiology, ecology, undergraduate research

INTRODUCTION

The Students as Scholars (SAS) program was developed to welcome first-year students into college with a high level of mentoring, engagement, and community. The persistence of undergraduates in STEM majors is a focal point for improving the quality and diversity of graduates entering into scientific disciplines. Approximately 50% of students who declare a STEM major when entering college will change majors before graduation, with greater than 60% of underrepresented students changing majors (National Academies of Science Engineering and Medicine [NAS], 2017; Estrada et al., 2018). This "leaky pipeline" has been a historical focus of scholarly assessment and improvement of higher education in STEM, working to identify high impact practices that improve persistence in STEM majors. One focal point for this scholarship has been the potential benefit of undergraduate research experiences (UREs), which serve the central learning outcomes defined by the American Association of Colleges and Universities. These outcomes focus upon developing both intellectual and practical skills, applied learning, and both personal and social responsibility. A 2017 report by the National Academies of Science defines a URE program as one that provides students with authentic research experience, invested mentoring, a collaborative environment, opportunities to learn and interpret data, and improved skills in communicating results (National Academies of Science Engineering and Medicine [NAS], 2017). Noting the potential impact of URE programs, universities are investing more in authentic research experiences for STEM students. Programs include full-time summer undergraduate research programs, course-based undergraduate research experiences (CUREs), and part time participation in research during the academic semester. These opportunities vary widely with regard to student experience and expectations, but share a recognition that the active process of discovery is an authentic and compelling introduction to the heart of STEM disciplines.

Curriculum development to improve the experience of a STEM major will typically examine student engagement over 4 years: from initially being an observer of the research process to gradually becoming an active research scholar, displaying mature hypothesis formulation, experimental design, and professional engagement (Estrada et al., 2016; Wilson et al., 2018). Though challenging to independently assess, influential factors in student persistence include scientific efficacy, professional identification in community, close mentoring, and authentic research engagement early in the collegiate experience (Graham et al., 2013; Light and Micari, 2013; Estrada et al., 2018). UREs provide student experiences that improve persistence in the sciences, especially when provided during the first 2 years of college (Jones et al., 2010). Dating back to 1988, the Meyerhoff Scholars Program at the University of Maryland-Baltimore offers a carefully developed 4 year strategy for students pursuing research-oriented careers. The program includes research experience beginning in the first-year, partnered with early engagement of a scholarly community, summer research interships, advising, and sustained mentoring. The success of this program in improving student diversity on campus, and achieving strong STEM persistence with these populations has gained wide attention. In 2019, the "Meyerhoff Model" was used to develop similar programs at UC San Diego and UC Berkeley, funded through the Chan Zuckerberg Initiative. Though requiring a heavy institutional investment, including faculty time, these types of programs are successful in improving the persistence of under-represented populations of students.

The SAS biology program at Pepperdine University was developed in response to this evidence of the importance of student experience in the first-year and with the prior success of the Keck Scholars program for students majoring in diverse disciplines. Prior to these programs, student engagement in research during their first-year was limited, and often delayed until their junior year. This was especially the case for students requiring foundational coursework in microbiology, biochemistry, and cell biology. During their first-year in college, the first-year SAS program integrated student mentors, dedicated class time on specific foundational concepts, and applied a methodical approach to project design. With regard to the projects defined here, partnership between a first-year class and an upper division course in microbiology and plant physiological ecology was essential to project success. Students in the higher-level class brought skill sets in microbiological laboratory techniques, field portable gas-exchange, and data analysis which were essential to project success. In this manuscript, we focus upon SAS program content at Pepperdine and note that Whittier College was a partner in this work, though their method of enrolling students in first-year seminars was slightly different.

Program Structure

The SAS program was developed within the context of first-year seminar classes, which integrate academic content with orientation topics, dialogue on collegiate best practices, intensive writing, and content on career exploration. Faculty teaching these classes met regularly during the school year to discuss class progress and best practices. Incoming biology majors enrolled in either a SAS course, or another first-year seminar class. It should be noted that all biology majors were encouraged to register for a SAS class as their first-year seminar. In the first year of the program, we had sufficient capacity (50–60 students) for

all majors. In years 2 and 3, growth in the student population resulted in an inability of these classes to fully accommodate all majors, and thus some students enrolled in other first-year seminar classes. Comparisons in STEM persistence between the SAS and non-SAS students were recorded. We note that though capacity was a significant cause for biology majors enrolling in non-SAS classes, there is the potential that some students pre-selected against research, which would be predictive of a weak affinity for STEM disciplines. Classes met for 3 h of lecture time with an additional 3 h of time allotted for research in a teaching laboratory, greenhouse, and field sites. Biology students not taking a SAS first-year seminar class were enrolled in a biology colloquium class focused upon introducing the biology major to new students via topical content and group assignments. Early in the semester, all SAS courses introduced the scientific method and explored some research data from within their content area of study. An institutional volunteer day for community outreach and service was used by each SAS class to engage with resource managers of private, city, and state parks. The microbiological data collected ranged from assessing soil salinity, soil respiration, and plant water status in a local park struggling with salinity in the soil, to simple coliform counts within the Malibu watershed in areas experiencing vegetation dieback. The data collected was reported back to park managers during a symposium event hosted by the SAS classes (**Figure 1**). This integration of student training with public service was a helpful first step in building student teamwork and motivation for extending their work to full research projects. We also note student excitement that local park managers were appreciative of the data generated, information gleaned, and recommendations provided.

In the second half of the semester, students began to work in research groups, with an initial focus upon refining a research question. Groups met to discuss ideas, to read research papers, to engage with experienced research students from the biology major (class mentors), and ultimately to offer a research proposal to the class. This proposal was presented orally, and subsequently received feedback from the class, the research mentors, and from the professor. After feedback was received, students refined their project and submitted a final proposal for approval by the faculty member. Most projects required at least 6 weeks of data collection to address the research question. For microbiological projects, there was a range of work from assessing biodiversity in soils using ecoplate technology, quantification of coliform levels in local parks and lagoons, evaluation of soil respiration along a salinity gradient, and genomics-based assessment of soil microbial diversity. Each class had a timeline for concluding data collection and moving into analysis and preparation of a summative poster. Faculty recognized that student groups would be in varied stages of project completion, nonetheless, all groups developed a poster to tell the story of their project. The concluding poster session included all first-year biology students, administrators, faculty, upperclassmen from the biology major, and campus guests. Through NSF funding, students were able to submit a small grant proposal that could provide funding for further research the following summer. These proposals were competitively reviewed and awarded for a cohort of approximately 8 students/summer. We note that these SAS classes generated a significant number of students who continued their research engagement throughout their undergraduate training.

Research Project Examples in Microbiology

As the SAS program matured, the faculty became more experienced with guiding first-year students to projects that were appropriate. The program goal was to develop projects that asked scholarly research questions and were practical in the context of first-year students and their available time. The importance of the student mentors was clearly realized during project design, students appreciated having experienced research students available to help them interpret the literature, and to develop research approaches. Faculty expressed the importance of hosting class projects with some overlapping techniques and focus, to simplify the work of research support and to build interest between research groups.

Examination of Water Quality in the Malibu Community

Upon initial offering of this program, microbiological projects focused upon water quality testing and soil microbial diversity. The Malibu community has local parks, creeks, and marine estuaries that each have unique dynamics of watershed function including water contamination risks and management decisions. Malibu's 6-acre Legacy Park was designed to reduce microbial contamination from local water flows using a naturalized stream channel and vegetated detention basin (Brager and Thorsen, 2011). Student groups met with the park managers to discuss the park design, and challenges with water management at the park. Students designed a project focused upon measuring coliform levels in part waterways and surge channels leading to the ocean as well as from groundwater at the site. Some samples were further evaluated by 16S rRNA gene sequencing to identify the microbes present. The project revealed a uniform distribution of coliforms throughout the park waterway, rather than a decline in concentration at the outflow point. Students interpreted this to be caused by higher than expected rates of water flow, and entry of water into the park from unanticipated locations. Though limited in scope, this provided the students with some experience with molecular biology techniques and genetic analysis. We note another project in which students were evaluating the filtering properties of marine estuaries. They measured coliform levels weekly along a local creek leading to an estuary adjacent to the Pacific Ocean. Interestingly, they identified a region of the creek that displayed a clear spike in coliform (including *E. coli*) counts in weekly tests. Investigation of that sampling by the students revealed a hidden encampment of homeless people adjacent to the creek and evidence of human waste along the creek margins. These microbiological projects provided technical engagement, insight into sampling methods, microbial culture experience, and an introduction to microbial identification via 16S rRNA gene sequencing.

Analysis of Soil Microbiome and Respiration Through Cross-Course Collaborations With SAS First-Year Seminars

The faculty/students of one SAS class established a cross-course collaboration with a class focused upon genomics analysis which is titled, The Application of Genomic Strategies in Human and Microbial Diversity. This cross-course collaboration enabled research projects evaluating soil respiration and soil microbiome composition along a soil salinity gradient at Legacy Park (**Figures 1A,B**). Dr. Stephen Davis' research group at Pepperdine has contributed significantly to the understanding of environmental stresses on chaparral vegetation (Venturas et al., 2016; Jacobsen et al., 2018). However, the effect of environmental stress on the interaction between the soil microbiome and Chaparral plant species represents an understudied research area and a unique opportunity to engage undergraduates in novel genomics and ecological research.

The objectives of this collaboration were threefold: (1) to introduce freshmen to the scientific process in preparation for engagement in an authentic research experience, (2) to introduce Pepperdine upperclassman to next-generation sequencing strategies and analysis, and (3) to provide scientific information for the wise management of Malibu's Legacy Park and the park's efficacy in soil water purification for the city of Malibu. We also wanted to determine the cause of selective plant mortality in Legacy Park for improved landscape management. Student's tested the hypothesis that plant dieback patterns were associated with heterogeneity in soil salinity, soil microbial composition, and soil respiration. The collaborative project was especially impactful on beginning freshmen students who had just completed their first 3 weeks of college on the Malibu campus of Pepperdine University. First-year students experienced the scientific process of hypothesis formulation, experimental design, data reduction, interpretation of results, and presentation of their research findings before city managers of Legacy Park at a symposium and final poster session (**Figures 1C,D**).

Analysis of Soil Salinity, Respiration, and Microbial Composition

The soil microbiome is a key contributor to the health of plants (Dubey et al., 2019). One way in which the microbiome directly influences surrounding plants is through the liberation of carbon dioxide through the process of respiration (Gougoulias et al., 2014). Consequently, shifts in the soil microbial environment could drastically alter atmospheric CO_2 (Gougoulias et al., 2014). There is evidence that exposure to environmental stress alters

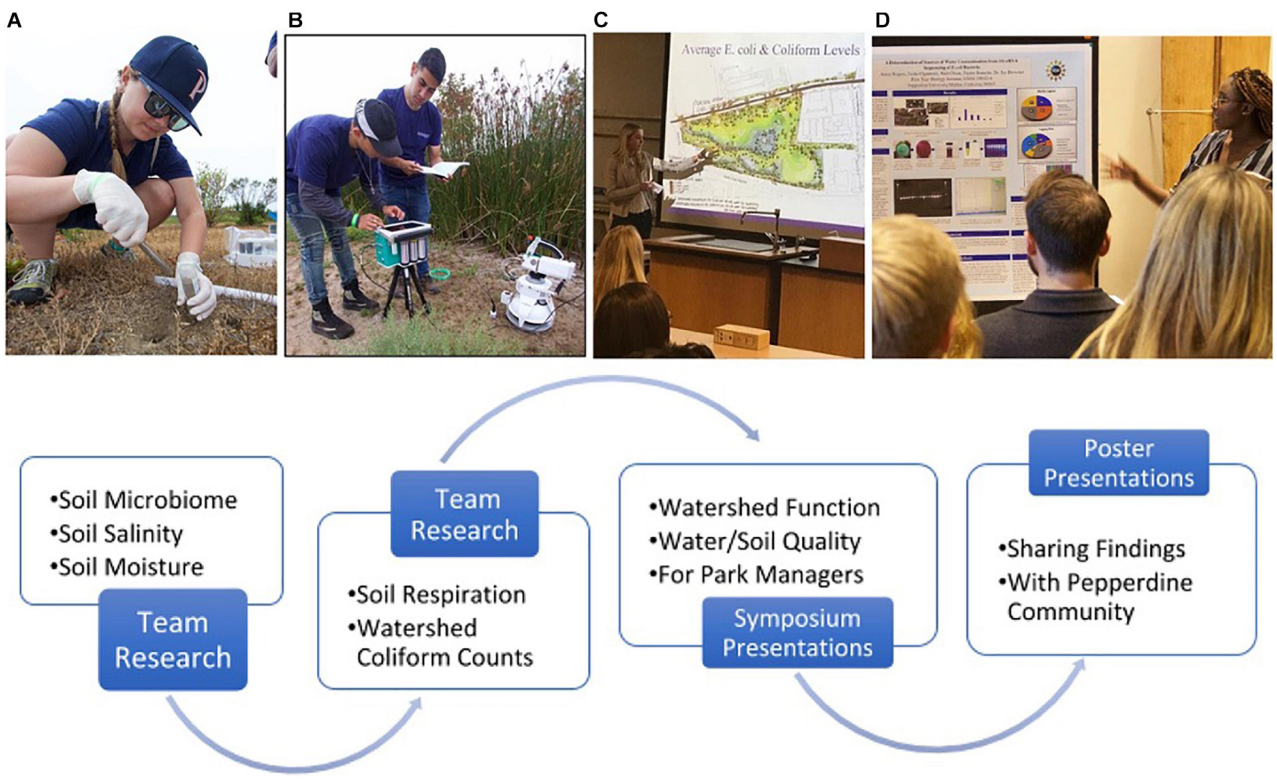

FIGURE 1 | SAS-Program Activities: Pepperdine students **(A)** collecting soil samples for microbiome, salinity, and moisture analyses; **(B)** measuring soil respiration with a field-portable CO_2 flux chamber and gas-exchange system; **(C)** giving symposium presentations on water and soil quality for environmental managers of coastal wetlands; and **(D)** sharing research findings in a final poster session for Pepperdine University's community of learners, attracting over 120 participants. Photos by SD Davis.

the diversity of the soil microbial community (Hinojosa et al., 2016; Li et al., 2019; Whitman et al., 2019), however literature is lacking on the effect of environmental stress on Chaparral-microbiome interaction.

SAS students along with senior-level upperclassmen enrolled in the genomics course collected soil respiration data and samples for bacterial microbiome analysis from two sites in Malibu's Legacy Park (**Figure 2A**). The two sites from which these data were collected represented a range of environments differing in dryness and salinity: the hilltop site was farthest from the creek bed, near a working irrigation system, while the valley site was near a moist creekbed. Respiration and salinity were measured in dry and wet soil from both sites, while microbiome composition was analyzed solely in the dry hilltop and wet valley sites (**Figures 2**, **3**).

For analysis of microbiome composition, students first isolated metagenomic DNA from soil samples collected from each site using the QiaAmp DNA Stool MiniKit (Qiagen). An additional bead beating step was added to ensure proper isolation of both gram positive and gram-negative bacteria. Metagenomic DNA was then submitted to the UC Davis Host Microbe Systems Core for library preparation and targeted amplicon sequencing of the 16S ribosomal RNA gene. Samples were subjected to a nested PCR for library preparation prior to sequencing. Specifically, from the metagenomic DNA, the full length 16S rRNA gene was amplified prior to a second PCR during which the V4 region of the 16S gene was amplified, enhancing the ability to detect bacterial taxa in the samples. Prior to analysis by the students, samples were pre-processed using DADA2 and amplicon sequence variants (ASVs) were assigned taxonomy against the SILVA database (Callahan et al., 2016). Students then completed analysis of the soil microbiome using the R-based packages (Phyloseq and ggplot2), comparing relative abundances of bacteria at the phylum level (McMurdie and Holmes, 2013; Wickham, 2016; **Figure 2**). For relative abundance analysis, raw read counts are transformed to percent abundance, wherein the percent of each bacterium is calculated relative to the total number of reads per sample.

Students measured soil volumetric water content using time domain reflectometry, soil temperature with a thermistor, and soil salinity with a precision electrical conductivity sensor (Decagon 5TE sensor with ProCheck Meter, Decagon Devices, Inc., Pullman, WA). Electrical conductivity was converted to salinity in dS m^{-1} using a ratio of 10 g distilled water to 10 g soil in a 1:1 ratio and correlation to salinity measured by the saturated paste method. Students measured soil respiration using two separate soil CO_2 flux chambers coupled to field portable gas-exchange systems (Li-6800, LiCor, Inc., Lincoln, NE). Students used three replicates at each sampling site after they installed 12 soil collars 3 days prior to initial sampling. Mean values were compared among treatments using a one-way ANOVA, followed by Fisher's least significance tests, where appropriate (**Figure 3B**).

Soil salinity and respiration were measured in dry and wet soil from hilltop and valley sites in Legacy park (**Figures 2A**, **3**). Soil salinity was highest at the wet valley site. This heightened salinity correlated with decreased soil respiration (relative to the hilltop sites) and increased bacterial diversity (**Figures 2**, **3**). Bacterial community analysis from metagenomic soil DNA revealed increased abundances of Proteobacteria and Bacteroidetes in the valley site, taxa which have been previously associated with high soil salinity (Canfora et al., 2014). In the hilltop site, we note an increased abundance of Cyanobacteria. Soil biocrusts containing cyanobacteria have been reported to occur widely in undisturbed soils, including hilltops, of coastal sage scrub communities, similar to those of Legacy Park, in Malibu (Hernandez and Sandquist, 2011). One study reports a correlation between soil respiration rates and the abundance of certain classes of Proteobacteria (Li et al., 2011), suggesting this taxon could be contributing to the variation in soil respiration among the Legacy Park sites.

Though preliminary, the soil microbiome analysis provides key insight into the Legacy Park ecosystem. Coupled with the assessment of soil respiration and salinity, students are able to draw conclusions about the connectedness of the soil microbial community and the surrounding Chaparral environment. Further, this study highlights the feasibility of such collaborative research, conducted entirely by undergraduate students. Notably, soil respiration and salinity were analyzed by first-year undergraduates, guided primarily by their peer mentors.

In the future, additional samples can be collected from these sites in Legacy park to validate these findings. Other projects related to these soil collection sites include analysis of the soil mycobiome via sequencing of the internal transcribed spacer region (ITS sequencing) and/or quantification of specific bacterial and fungal species from soil using quantitative PCR. In addition, SAS students in the Davis and Stiemsma research groups have also begun using these techniques to study the effect of wildfire on soil microbial communities using samples collected after the Woolsey Fire of November 2018.

Genomics Datasets as Teaching Tools in SAS and Other Life Sciences Courses

Interest in microbiome research continues to be received with great enthusiasm by the scientific community. Beyond the study of microbiome composition, health research and environmental ecology are examples of fields where the integration of microbiome analysis with other genomic strategies is utilized to assess host-microbiome interactions (Jansson and Hofmockel, 2018; Awany et al., 2019). The growing application of next generation sequencing has also significantly enhanced the roles of bioinformaticians and computational biologists in fields such as these, highlighting the need to engage students in these subjects early on in their undergraduate careers.

As represented by this cross-course collaboration with the SAS program at Pepperdine, studying the microbiome using next-generation sequencing is relatively easy to integrate into the classroom. The majority of computational tools (namely R) used to analyze microbiome sequencing data are open-source and free for users, decreasing the need to purchase expensive licenses for software or to implement additional laboratory fees to cover the cost of conducting these analyses. Further, the continued decrease in sequencing costs and streamlining of the wet and dry laboratory procedures to conduct microbiome analysis will

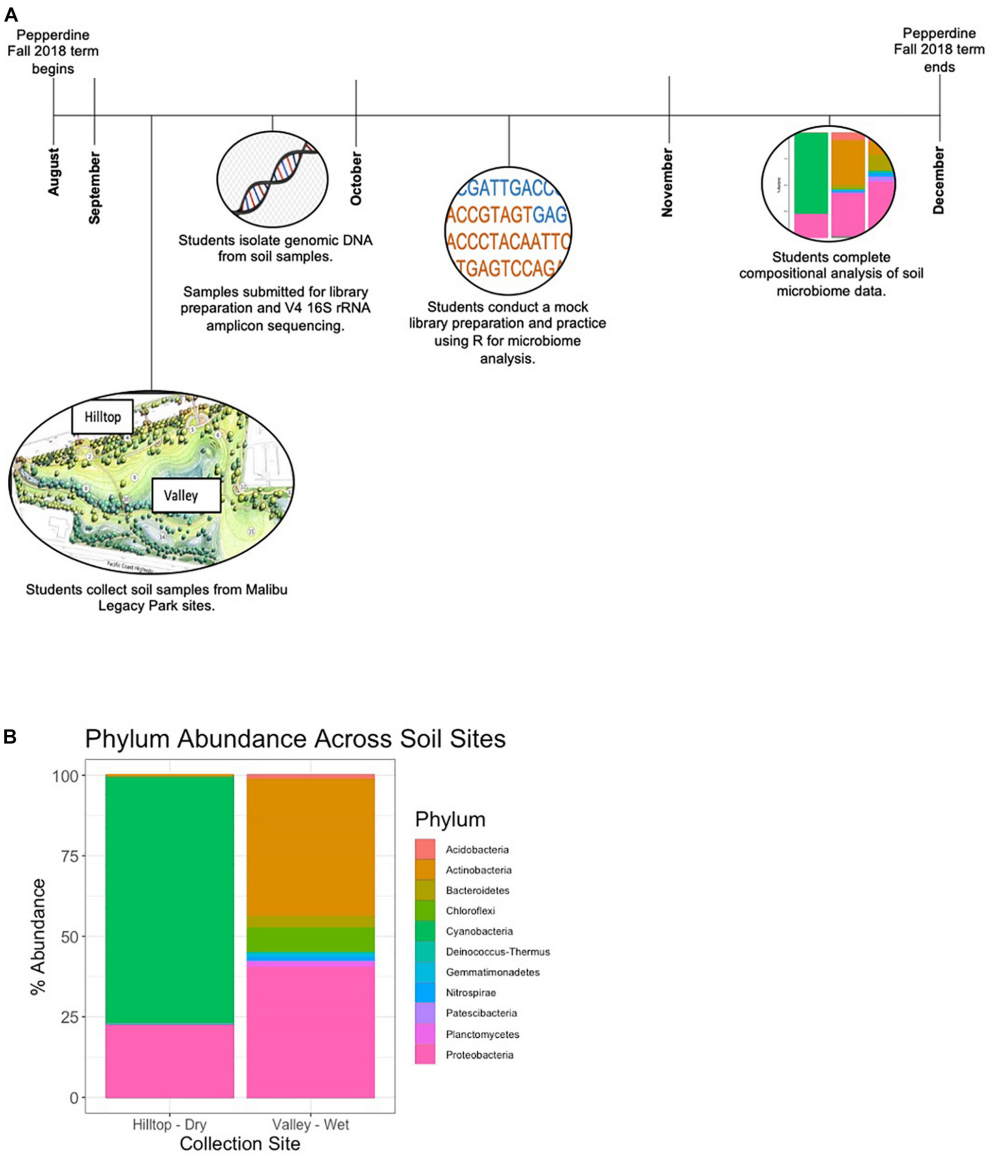

FIGURE 2 | Soil microbiome analysis. **(A)** Timeline of the student-led soil microbiome sequencing project. **(B)** Relative bacterial phylum abundances across Legacy Park sites.

significantly enhance the ability to conduct this applied research in the classroom.

Beyond our introduction of next-generation sequencing technology to and analysis of the human microbiome by this student group, we have also established lab modules that incorporate analysis of these datasets in other courses (e.g., Microbiology and Genetics). These lab modules serve to introduce students to the field of genomics and the use of R for microbial genomics analysis. Combined with more student-generated sequencing datasets, these modules will be key in expanding genomics in the SAS program. Further, the study of personal microbiomes in the classroom was reported to increase student engagement in genomics-based coursework (Weber et al., 2018) and represents one additional type of microbiome analysis that could be implemented into an SAS first-year seminar, among other life science courses at Pepperdine.

Concluding Poster Session and Follow Through

At the conclusion of each SAS program, student research teams presented their findings at a reception and poster session. This was well attended by Pepperdine students, faculty, and administrators. Students regarded this event as intimidating, but an encouraging conclusion to their first semester of college. To date, these projects have resulted in 16 presentations at regional conferences, such as the Southern California Conference for Undergraduate Research, and 15 presentations at annual

meetings of national scientific conferences such as the 113th Botanical Society of America meeting, 103rd Ecological Society of America meeting, and 58th American Society of Cell Biology meeting.

Adapting SAS to the COVID-19 Environment

Notably, COVID-19 has dramatically altered the ability to conduct research at universities across the United States. Though wet-laboratory work may be challenging, we have highlighted ecological and genomics projects in this report that could be easily adapted to a virtual environment. Firstly, the collection of soil, water, and plant samples/assessments takes place outdoors, which limits aerosol transmission of microorganisms. Secondly, with regard to genomics research, once samples are collected, DNA can be isolated in a limited-personnel lab (maintaining social distances guidelines). Once DNA is isolated, the remaining analyses are computational, meaning the students can complete their analyses from anywhere with a sufficient internet connection. Pepperdine has also recently launched a secure server with access to R-studio for students enrolled in classes or research, further facilitating a virtual research environment.

Assessment of the SAS Program Highlights Increased Retention of Students in STEM Disciplines

The SAS program has been highly successful at Pepperdine. Throughout the program, students have participated in assessment through focus group discussions and pre/post program surveys. In the spring of 2020 the first cohort of SAS students were in our group of graduating seniors. Their feedback on exit surveys was highly supportive of the program, with several students noting the SAS program as critical in their decision to remain in sciences, or even at Pepperdine. We have collected data on student persistence in STEM disciplines since the establishment of the SAS program in 2016. Persistence in a STEM major was examined from student records at the conclusion of their third semester of Pepperdine enrollment. In the Pepperdine SAS programs of 2017 and 2018 our STEM discipline attrition rate averaged 15.3% while attrition of non-SAS biology students averaged 30.2% (**Figure 4**). Alternatively stated, we report 15% increased retention of students in STEM majors during their first year in college, reducing typical attrition by one-half. Also of note, Pepperdine assessed all first-year students in 2017, examining characteristics of student care, health, and connection at Pepperdine. As all first-year students participate in a first-year seminar class, we found this an opportunity to compare the Pepperdine SAS students to all other first-year students. When asked to rate the ease at which they have made friends during their first semester, 54% of the SAS cohort responded "very easy" while only 25% of students enrolled in other categories of first-year seminars responded similarly. When asked about the frequency of feeling loneliness, 9% of SAS students responded "often" or "all the time" contrasted with 19% of students in all other first-year seminars. When asked if their first-year seminar helped the student to transition into college, 40% of SAS students responded "a lot" while the average response from other first-year programs was 27%. This analysis of first-year seminars provided good support that this course structure was providing community to our incoming students, and an improved integration into the campus environment.

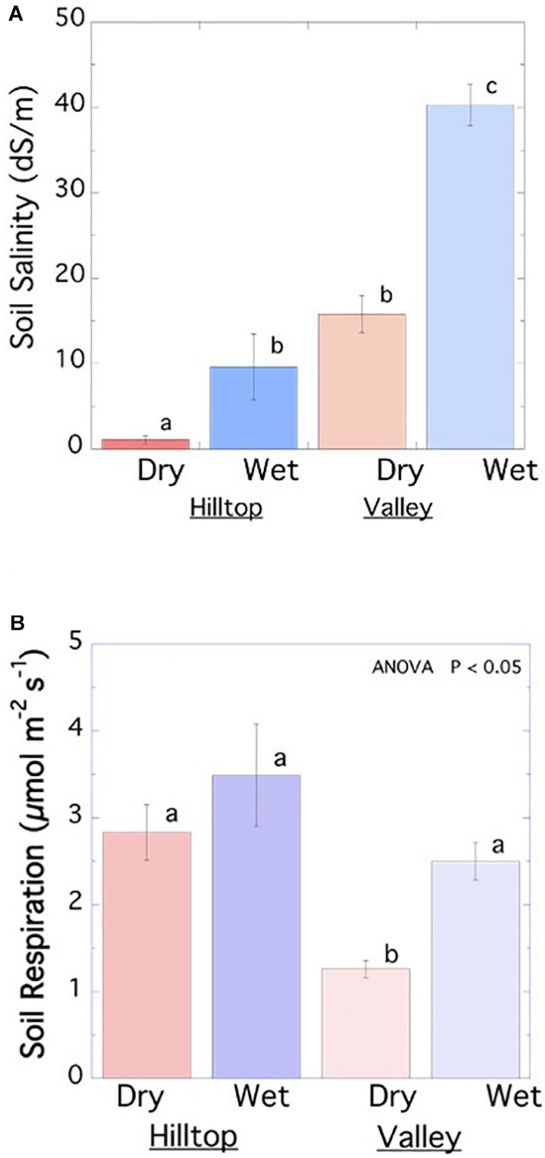

FIGURE 3 | Analysis of soil **(A)** salinity and **(B)** respiration in Legacy Park at hilltop low soil salinity and high soil respiration sites compared to valley bottom sites experiencing high salinity and low respiration. Differences between letters on bar graphs indicate significant difference between treatments by one-way ANOVA followed by a Fisher's least significant difference test at $P < 0.05$. Error bars on symbols represent ±1 SE, $n = 3$.

An annual process of SAS assessment for both Pepperdine and Whittier College students was managed by the WestEd

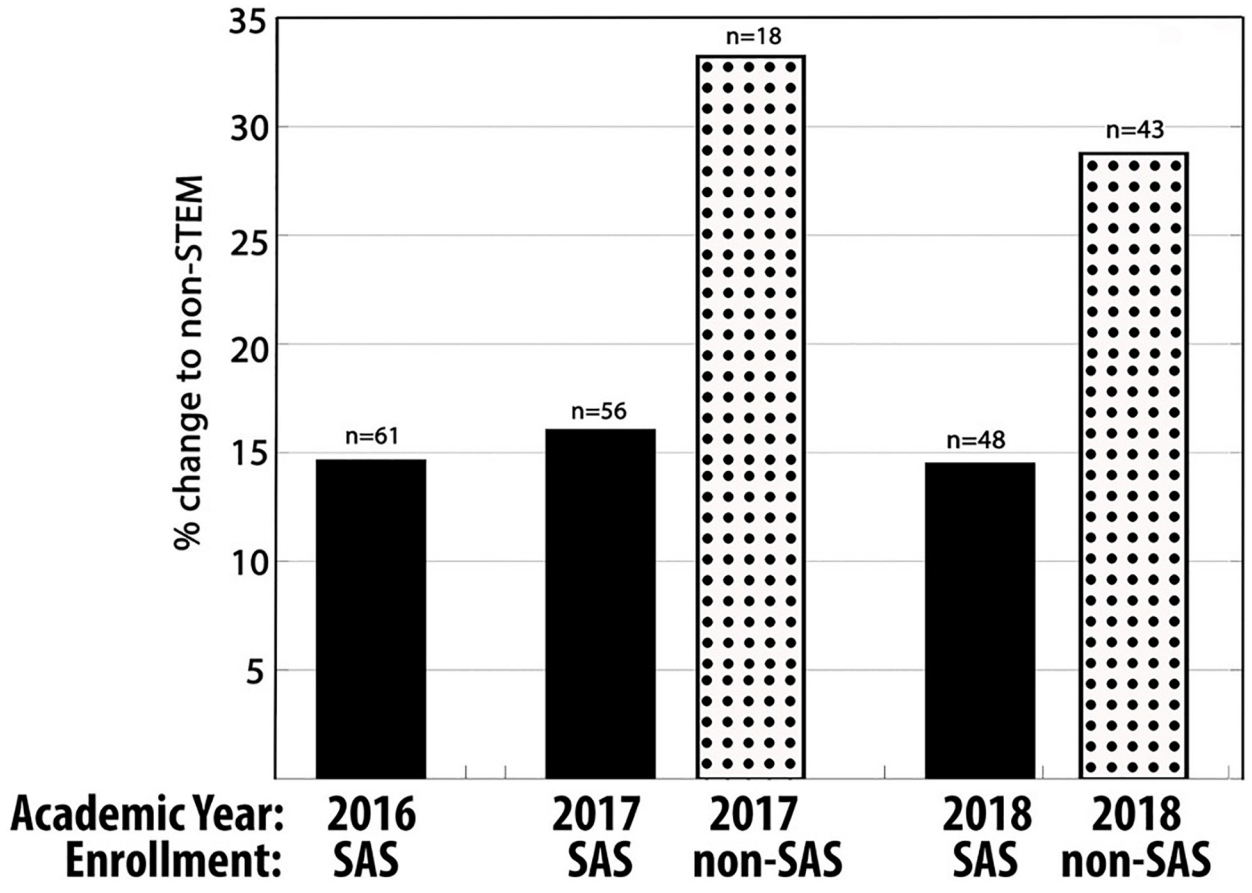

FIGURE 4 | Retention of SAS and non-SAS students in STEM disciplines in 2016, 2017, and 2018. There are no data for non-SAS students in 2016 due to limited sample size. These data represent attrition rates for first-year biology students at Pepperdine University. These data were scored by declared major at the conclusion of the third semester of enrollment. All students assessed entered Pepperdine as biology majors.

educational consulting group, and the data were reported in combined format. We have provided some key points of information from that assessment, using data from the 2017 SAS groups at both institutions. The data shown are consistent with assessments from other years. Please note that full participation in this assessment was a requirement of program participation. Student self-efficacy in research was assessed on four dimensions of research ability; (1) designing experiments, (2) competency with instrumentation, (3) data analysis, and (4) communicating results). When comparing pre/post program participation, student self-efficacy increased significantly from an average of 2.4 prior to completing an SAS course to 3.7 after completing an SAS course (**Figure 5A**). These self-assessments suggest an overall level of increased confidence in conducting STEM research amongst SAS scholars. Questions focusing upon student attitudes about their science major examined factors that are likely to predict persistence. The majority of SAS students (85%) report that their involvement in an SAS course increased their interest in a science major either "somewhat' or "a lot" (**Figure 5B**). Also, 73% of SAS students stated that their enrollment in an SAS course influenced them to keep their choice of major. These two data points address a key goal of the program, to improve student persistence through the critical first-years of college. Beyond persistence in STEM disciplines, participation in the SAS program was key in enhancing student interest and efficacy in research. Specifically, 85% of students reported an interest in pursuing other research opportunities during their undergraduate career and 73% of SAS students report that they are confident they will present their research at a research conference in the near future. The influence of student mentors and faculty mentors in their academic success and transition into college life were perceived as extremely valuable (**Figures 5C,D**). Collectively, these data highlight the importance of the SAS program in retaining STEM majors and enhancing student learning at the undergraduate level. Students who participated in the SAS program engaged more with their peers, gained confidence in their ability to apply life science content through research, and showed greater likelihood of

A

Research Skills	Mean Pre-Program	Mean Post-Program	P-Value
Designing Experiments	2.4	3.7	<.001
Competency with Instrumentation	2.1	3.5	<.001
Data Analysis	2.5	3.7	<.001
Communicating Results (oral)	2.4	3.7	<.001

Scale: 1= not at all, 2= slightly, 3= somewhat, 4= moderately, 5= a great deal

B

How much did the SAS course:	Not at All	Slightly	Somewhat	A lot
Increase your interest in a science major?	7%	8%	40%	45%
Influence you to you to **keep** your choice of major?	15%	10%	31%	43%
Influence you to **change** your choice of major?	67%	15%	11%	7%

C

FYS Student Asked Mentor for help with:	Never	Rarely	Some-times	Often	Always
Assigned work for course	6%	14%	28%	38%	14%
Major or college life in general	7%	15%	28%	32%	18%

D

FYS Student valuation of mentor help with:	Not Useful	Weakly Useful	Somewhat Useful	Very Useful
Assigned work for course	1%	3%	29%	67%
Major or college life in general	1%	3%	35%	61%

FIGURE 5 | Exemplar SAS course assessment data generated from student surveys and focus groups. Data in panel **(A)** represents a comparison of student assessments of efficacy in the four dimensions of research ability before and after program participation. These paired measures were analyzed by *t*-test with statistical significance assigned to $p < 0.05$. Panels **(B–D)** offer representative data from program surveys offered upon conclusion of the program. Panel **(B)** displays student reporting of program influence upon interest and retention in a science major. Panels **(C)** and **(D)** reveal frequency and valuation of student access to a faculty mentor. All of the data shown represents feedback from SAS students in the 2017 program cycle with 90% student participation in assessment ($n = 110$). Pepperdine and Whitter college students are equally represented in these data.

continuing on in STEM for the duration of their undergraduate careers at Pepperdine.

CONCLUSION

The Students as Scholars program was designed in response to the research literature on undergraduate engagement and its impact upon the student success. Implementation of the program required the partnership of committed faculty willing to engage significant time in the mentoring of first-year students. In addition, the program required an infusion of funding from the National Science Foundation to support the cost of research supplies, stipends for student mentors, and research fellowships for students wishing to continue their research. A sustainable program will need to identify program components that accomplish key characteristics of the program, but within the available time from busy faculty, and within the budget available from the host institution. In our experience, this program provided clear improvement of student engagement, persistence, and satisfaction with their science major. The integration of scholarly work in microbiology was particularly appropriate due to the range of important questions that first-year students can experimentally evaluate, and the cohesion of traditional microbiological research with more advanced microbiome analyses linked to ecosystem services of fertile soil and clean water. Collaboration between a first-year seminar and an advanced elective course was surprisingly effective. The more experienced students were energized by the partnership and proud to display their expertise and to train younger students. Similarly, student mentors established healthy relationships with their class and played an important role in project design and execution. As faculty became convinced of the benefits for students, recruiting participating faculty became easier. We are working to modify the program to implement the program without external funding, and to expand the program beyond students majoring in biology. The SAS program has provided clear support for institutional investment in the first semester of college to ensure students arriving with a disciplinary interest have the opportunity to truly experience the merits and community available to them in that particular STEM discipline.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA649789.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors contributed to the data analysis and writing of this manuscript.

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REFERENCES

Awany, D., Allali, I., Dalvie, S., Hemmings, S., Mwaikono, K. S., Thomford, N. E., et al. (2019). Host and microbiome genome-wide association studies: current state and challenges. *Front. Genet.* 9:637. doi: 10.3389/fgene.2018.00637

Brager, R. L., and Thorsen, J. (2011). *Malibu's Cleaning Machine*. Reston, VA: ASCE.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583. doi: 10.1038/nmeth.3869

Canfora, L., Bacci, G., Pinzari, F., Lo, P. G., Dazzi, C., and Benedetti, A. (2014). Salinity and bacterial diversity: to what extent does the concentration of salt affect the bacterial community in a saline soil? *PLoS One* 9:e106662. doi: 10.1371/journal.pone.0106662

Dubey, A., Malla, M. A., Khan, F., Chowdhary, K., Yadav, S., Kumar, A., et al. (2019). Soil microbiome: a key player for conservation of soil health under changing climate. *Biodivers. Conserv.* 28, 2405–2429. doi: 10.1007/s10531-019-01760-5

Estrada, M., Burnett, M., Campbell, A. G., Campbell, P. B., Denetclaw, W. F., Gutierrez, C. G., et al. (2016). Improving underrepresented minority student persistence in STEM. *CBE Life Sci. Educ.* 15:es5. doi: 10.1187/cbe.16-01-0038

Estrada, M., Hernandez, P. R., and Schultz, P. W. (2018). A Longitudinal study of how quality mentorship and research experience integrate underrepresented minorities into STEM careers. *CBE Life Sci. Ed.* 17:ar9. doi: 10.1187/cbe.17-04-0066

Gougoulias, C., Clark, J. M., and Shaw, J. L. (2014). The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems: role of soil microbes in global carbon cycle: carbon tracking & agro-cosystem management. *J. Sci. Food Agric.* 94, 2362–2371. doi: 10.1002/jsfa.6577

Graham, M. J., Frederick, J., Byars-Winston, A., Hunter, A. B., and Handelsman, J. (2013). Increasing persistence of college students in STEM. *Science* 341, 1455–1456. doi: 10.1126/science.1240487

Hernandez, R. R., and Sandquist, D. R. (2011). Disturbance of biological soil crust increases emergence of exotic vascular plants in California sage scrub. *Plant Ecol.* 212, 1709–1721. doi: 10.1007/s11258-011-9943-x

Hinojosa, M. B., Parra, A., Laudicina, V. A., and Moreno, J. M. (2016). Post-fire soil functionality and microbial community structure in a Mediterranean shrubland subjected to experimental drought. *Sci. Total Environ.* 573, 1178–1189. doi: 10.1016/j.scitotenv.2016.03.117

Jacobsen, A. L., Pratt, R. B., Alleman, D., and Davis, S. D. (2018). Post-fire ecophysiology of endemic chaparral shrub seedlings from Santa Catalina Island. Southern California. *Madroño* 65, 106–116. doi: 10.3120/0024-9637-65.3.106

Jansson, J. K., and Hofmockel, K. S. (2018). The soil microbiome — from metagenomics to metaphenomics. *Curr. Opin. Microbiol.* 43, 162–168. doi: 10.1016/j.mib.2018.01.013

Jones, M., Barlow, A. T., and Villarejo, M. (2010). Importance of undergraduate research for minority persistence and achievement in biology. *J. High. Educ.* 81, 82–115. doi: 10.1353/jhe.0.0082

Li, W., Niu, S., Liu, X., and Wang, J. (2019). Short-term response of the soil bacterial community to differing wildfire severity in Pinus tabulaeformis stands. *Sci. Rep.* 9:1148.

Li, Y., Wang, L., Zhang, W., Wang, H., Fu, X., and Le, Y. (2011). The variability of soil microbial community composition of different types of tidal wetland in Chongming Dongtan and its effect on soil microbial respiration. *Ecol. Eng.* 37, 1276–1282. doi: 10.1016/j.ecoleng.2011.03.024

Light, G., and Micari, M. (2013). *Making Scientists: Six Principles for Effective College Teaching*. Cambridge, MA: Harvard Press.

McMurdie, P. J., and Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*8:e61217. doi: 10.1371/journal.pone.0061217

National Academies of Science Engineering and Medicine [NAS], (2017). *Undergraduate Research Experiences for STEM Students: Challenges and Opportunities*. Washington, DC: The National Academies Press.

Venturas, M. D., MacKinnon, E. D., Dario, H. L., Jacobsen, A. L., Pratt, R. B., and Davis, S. D. (2016). Chaparral shrub hydraulic traits, size, and life history types relate to species mortality during california's historic drought of 2014. *PLoS One* 11:e0159145. doi: 10.1371/journal.pone.0159145

Weber, K. S., Bridgewater, L. C., Jensen, J. L., Breakwell, D. P., Nielsen, B. L., and Johnson, S. M. (2018). Personal microbiome analysis improves student engagement and interest in Immunology, Molecular Biology, and Genomics undergraduate courses. *PLoS One* 2018 13:e0193696. doi: 10.1371/journal.pone.0193696

Whitman, T., Whitman, E., Woolet, J., Flannigan, M. D., Thompson, D. K., and Parisien, M. A. (2019). Soil bacterial and fungal response to wildfires in the Canadian boreal forest across a burn severity gradient. *Soil Biol. Biochem.* 138:107571. doi: 10.1016/j.soilbio.2019.107571

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer-Verlag.

Wilson, A. E., Pollock, J. L., Billick, I., Domingo, C., Fernandez-Figueroa, E. G., Nagy, E. S., et al. (2018). Assessing science training programs: structured undergraduate research programs make a difference. *Bioscience* 68, 529–534. doi: 10.1093/biosci/biy052

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Broader Impacts for Ecologists: Biological Soil Crust as a Model System for Education

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Biological soil crusts (biocrusts) are a complex community of algae, cyanobacteria, lichens, bryophytes, and assorted bacteria, fungi, archaea, and bacteriophages that colonize the soil surface. Biocrusts are particularly common in drylands and are found in arid and semiarid ecosystems worldwide. While diminutive in size, biocrusts often cover large terrestrial areas, provide numerous ecosystem benefits, enhance biodiversity, and are found in multiple configurations and assemblages across different climate and disturbance regimes. Biocrusts have been a focus of many ecologists, especially those working in semiarid and arid lands, as biocrusts are foundational community members, play fundamental roles in ecosystem processes, and offer rare opportunities to study biological interactions at small and large spatial scales. Due to these same characteristics, biocrusts have the potential to serve as an excellent teaching tool. The purpose of this paper is to demonstrate the utility of biocrust communities as a model system in science education. Functioning as portable, dynamic mini ecosystems, biocrusts can be used to teach about organisms, biodiversity, biotic interactions, abiotic controls, ecosystem processes, and even global change, and can be easy to use in nearly every classroom setup. For example, education principles, such as evolution and adaptation to stress, or structure and function (patterns and processes) can be applied by bringing biocrusts into the classroom as a teaching tool. In addition, discussing the utility of biocrusts in the classroom – including theory, hypothesis testing, experimentation, and hands-on learning – this document also provides tips and resources for developing education tools and activities geared toward impactful learning.

Keywords: biocrust, ecology, evolution, scale, patterns and processes, succession, classroom

INTRODUCTION

What Are Biocrusts?

Biological soil crusts (biocrusts; examples provided in **Figure 1**) are defined as a photosynthetic surface soil community living in and binding together the top millimeters of soil (Belnap and Lange, 2001; Weber et al., 2016). Within this community, the common major players are cyanobacteria, algae, bryophytes, and lichens, and these primary producers provide habitat and food for a diverse soil food web, including bacteria, fungi, diatoms, protozoa, nematodes, and microarthropods (Belnap and Lange, 2001; Darby and Neher, 2016). Biocrusts are widespread and common across the globe, covering about 12% of Earth's terrestrial surface (Rodriguez-Caballero et al., 2018a). They are present in all ecosystems where light reaches the soil surface and thrive in places where vascular plants are less dominant, which can include harsh environments, like polar deserts, high alpine zones, hyper-arid deserts, gypsiferous, or saline soils, as well as arid and semiarid regions. Biocrusts also are commonly a successional step in areas where disturbance has exposed the soil surface to light and thus you can see biocrusts in wetter ecosystems on soil that has been exposed (e.g., from road cuts, fires, or at the forefront of receding glaciers). Biocrusts contain all domains of life, supporting a complex soil food web, and performing all vital ecosystem functions, including primary production, nitrogen fixation, aggregation, carbon storage, and stabilization of soils and regulation ecosystem hydrology (Maestre et al., 2011; Darrouzet-Nardi et al., 2015; Barger et al., 2016; Weber et al., 2016; Chamizo et al., 2017; Faist et al., 2017; Eldridge et al., 2020). While biocrusts are vitally important, they are also vulnerable to changing climate and land use disturbances (Ferrenberg et al., 2015; Rodriguez-Caballero et al., 2018a). This mix of diversity of taxa, growth forms, function, and distribution, coupled with their responsiveness to our changing world (Reed et al., 2016), make biocrusts an ideal hands-on learning system for a variety of topics.

Biocrusts as a Model System for Teaching

For research, a model system is defined as a system that "displays a general process or property of interest, in a way that makes it understandable" (Vitousek, 2002). The utility of biocrusts as a model system in scientific studies has been clearly demonstrated (Bowker et al., 2014; Maestre et al., 2016) and provides a path forward for investigating the use of biocrusts as a model system in education. Biocrust research has increased exponentially in the last two decades, yet English language publications describing the use of biocrusts as a teaching tool have not followed. Nevertheless, educators have clearly stated that biocrusts would be of interest to use in their classroom and can act as an engaging tool to develop learning of core concepts. This interest and availability, whether field collected or grown in the classroom, coupled with biocrusts' portable size, communities that range from simple to complex, wide span of bright colors and shapes (**Figure 1**), and visually dramatic transformations after being wetted from desiccated dormancy, highlight the potential experiential learning power of using biocrusts in the classroom. Further, although biocrusts are small in stature, they play extremely important roles in a wide range of ecosystems and thus offer a logistically feasible option for hands-on student experience with a foundational biological community. The range in complexity of the different topic areas means that distinct aspects of biocrust biology and ecology could better suit a range of learning phases. For example, topics related to understanding biodiversity and examining ecosystem function

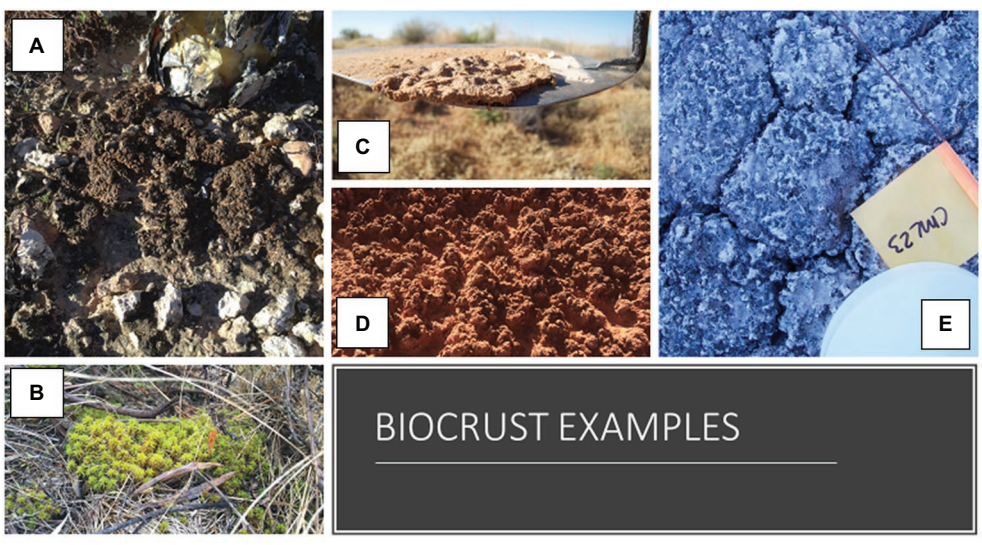

FIGURE 1 | Photographs of biological soil crusts in the field to highlight their variability in color and shape, as well as their ability to hold the soil surface in place. **(A)** Top left is a lichen dominated biocrust, **(B)** bottom left is a moss dominated biocrust, and **(C–E)** in the middle and on the right are examples of cyanobacteria dominated biocrusts.

can be used for university level students, while questions of taxonomy and morphology lessons could fit remarkably well into the new United States K-12 standards for Science, Technology, Engineering, and Mathematics (STEM) education called "three-dimensional learning."[1] This three-dimensional learning uses cross-cutting concepts that have applicability across all fields of science and provide real-world research practices to teach core concepts in Physical Science, Life Science, Earth and Space Science, and Engineering. Similar policies and standards can be met for similar age groups across Europe (European Commission/EACEA/Eurydice, 2019) and throughout other countries.

To use field collected biocrust samples in the classroom, local regulations must be met and guidelines for effective collection and cultivation of biocrust can be found in an ongoing biocrust restoration manual[2] and adapted for educational purposes specific to each classroom. Cultivating biocrust as a classroom project can be included into the curriculum with a terrarium of different collected components of biocrust communities (e.g., cyanobacterial filaments and moss spores) to then build on the concepts and activities discussed here. We provide suggestions for using biocrusts; however, many parallel learning tools apply using virtual efforts such as photographs or videos of filamentous cyanobacteria (e.g., https://3dmoss.berkeley.edu/). As a model system, there are numerous creative ways to use biocrusts in the classroom and in the field. We link four uses specifically positioned to highlight ecological and evolutionary principles taught across science-based curricula with associated activities in each (**Figure 2**).

[1]https://www.nextgenscience.org/three-dimensions
[2]https://anitaantoninka.wixsite.com/biocrustrestoration

POTENTIAL CONCEPTS USING BIOCRUSTS

Evolution

In plant and other evolution courses, the curriculum often starts with the cellular organisms that evolved earlier (e.g., cyanobacteria, then eukaryotic algae), and then works through to the spore producing non-vascular plants (e.g., mosses), before focusing on vascular plants. Those earlier life forms, cyanobacteria and algae are major components of biocrusts and can be easily identified through a hand lens or microscope, growing directly adjacent to later evolved organisms such as mosses and liverworts. Instructors can guide students through direct objectives to uncover evolutionary processes at the organismal level all within one petri dish (**Figure 2**). Students can learn about how studies using genetics can help use understand the multifaceted unseen microbial communities that are aiding in enhancing biocrust biodiversity (Liu et al., 2017; Van Goethem et al., 2019). Students can also use a hand lens or microscope to see the structure of an individual organism in order to learn taxonomy, evolutionary adaptations, and timelines in Earth's history (e.g., cyanobacteria ≥2 billion years prior to current time vs. moss ~450 million years prior). The communities can be linked to how evolution and ecology are intertwined and, while the different groups originated at such different times in history, show that they are still living together in similar space and time under current conditions. Biocrusts can also be incorporated

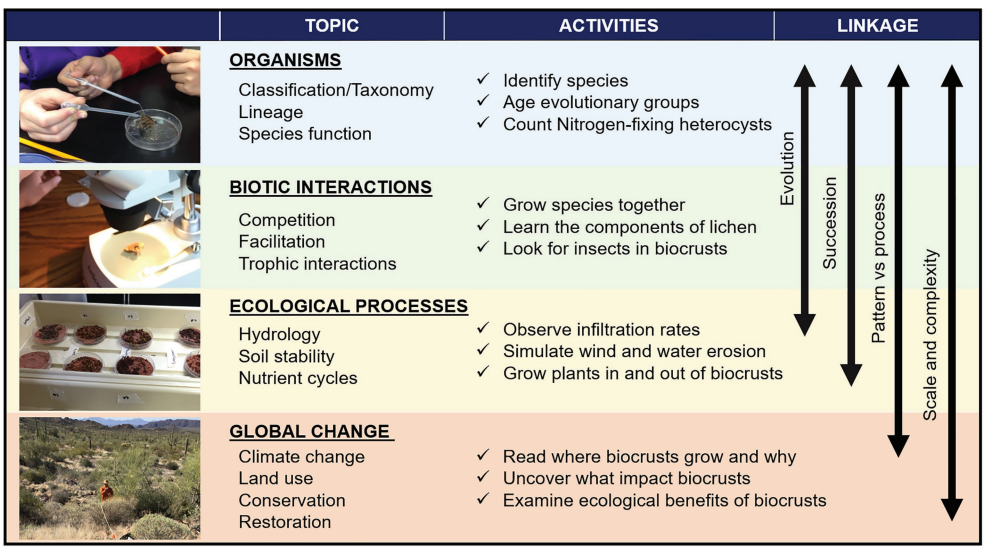

FIGURE 2 | Main study fields to be investigated in the classroom with subtopics, potential activities linked to them, and the underlying concepts linking the different topics. The photos illustrate from top to bottom a biocrust moss and giving a simple water addition to demonstrate how quickly biocrusts can hydrate and change form. Below is a microscope exercise where students identify the taxa within a small piece of biocrust with multiple species living in close proximity. Next is an experiment looking at how biocrust respond to different drought conditions, and finally a survey in the field to identify biocrust diversity, abundance, and impacts from land use.

into lesson plans about early colonization of land (Beraldi-Campesi and Retallack, 2016), as well as species and organismal relationships and interactions with each other (for example, facilitation and competition), which are central to ecology and evolutionary biology.

Succession

Succession refers to predictable changes in species composition over time as an ecosystem develops (primary succession; e.g., after a glacier recedes or lava cools) or recovers from a disturbance (secondary succession; e.g., after a forest fire or big flood). Biocrusts and their associated species demonstrate a general successional trajectory and predictable community assembly in both primary and secondary successional settings (Weber et al., 2016). As they quickly colonize soils, filamentous cyanobacteria can stabilize and fertilize the soil surface to then allow subsequent successional species, such as lichens and mosses, to colonize the soil surface. Observation of biocrust successional stages, whether in the classroom terrarium or in photographs, can be used to introduce actionable objectives relevant to all ecosystems at small to large scales, such as identifying similarities and differences in primary vs. secondary succession, and juxtaposing Clementsian climax models of succession vs. alternative ecosystem states (Bowker, 2007). Biocrusts are a great system to highlight specific organisms and their influence on the environment and thereby to underscore how changes to these communities can feedback to ecosystem processes and function through time.

Patterns and Processes

The ecological services and functions of biocrusts have been studied across systems (e.g., Belnap and Lange, 2001; Maestre et al., 2011; Weber et al., 2016; Faist et al., 2017; Rodriguez-Caballero et al., 2018b). In addition to identifying patterns of biocrust community assemblage, building the objective of understanding ecological processes can be gleaned through using biocrusts as engineering systems to test specific hypotheses and simulate system functions in the classroom. The random vs. organized patterns, or what we can see from a picture or directly through a microscope, can be used for students to document what they see at the organism level (**Figure 2**). In addition to documenting the magnified organisms and the patterns and shapes they create inside a petri dish, students can also learn about physiological and ecological processes.

The green color in the cells of biocrusts is due to photosynthetic pigments, performing the process of photosynthesis that is the foundation of most ecosystem functions and of the larger food web. Likewise, students can observe and quantify cyanobacterial heterocysts that are responsible for nitrogen fixation, which allows some organisms (those that can fix N_2) to access the enormous pool of nitrogen in the atmosphere that is unavailable to most organisms. These nitrogen-fixers then release this nitrogen (for example, when they die and decompose) and, because all living things need nitrogen, this process – nitrogen fixation – sustains life on Earth. All this together and other unmentioned examples allow students to see evidence of both the small-scale patterns (i.e., what they can see from a photo or under a microscope) and processes of biocrusts and of the larger ecosystem level processes they control. From here, the function of a single organism can be connected with the provision of nutrients across a landscape, which again demonstrates concrete links between pattern and process.

Another ecosystem process well-illustrated by biocrusts is soil erosion and its control. Filamentous cyanobacteria, mosses, and lichens all can bind together the soil surface and greatly reduce erosion (Chamizo et al., 2017; Kheirfam et al., 2017). A tangible demonstration of the erosion concept can be achieved with hands-on activities in which students make a pair of petri dishes or trays; one with dry biocrust, and a second with a loosely packed soil. The students can than test these petri dishes with wind or water forces; using a small fan (or even blowing on the dishes), or simulating rain (e.g., with a watering can). In both cases, the students can observe how biocrusts bind the soil and prevent wind and water erosion. Biocrusts can link these processes to the community of organisms within that petri dish to help students conceptualize how biocrust filaments and plant roots function in similar ways to protect soil from erosion (**Figure 2**).

Scale

You cannot always take your classroom out into the world, but you can bring the world into your classroom with biocrusts. The biocrust-filled petri dish, terrarium containing living biocrust communities, or photographs of multiple dynamic species that students can observe are true ecosystems at the small scale, with all major trophic levels and major ecosystem functions (Bowker et al., 2014, 2016; Maestre et al., 2016). This micro-model of a terrestrial ecosystem can be used to introduce systems thinking and build on the objective of developing a deeper understanding of how scale can influence processes on the landscape. Similarly, this micro-ecosystem can be scaled all the way to global processes and global change drivers. This could include discussions of how and why different biocrusts live in different climates (Samolov et al., 2020), how biocrusts interact with the living and abiotic landscapes around them (Rodriguez-Caballero et al., 2019), and how their activities at the small scale could be affecting function at the global scale (Ferrenberg et al., 2017).

DISCUSSION

In addition to the direct interest from teachers, lesson plans and outreach efforts that use biocrusts can meet broader impact goals of many granting agencies. Education and outreach about biocrusts is at an all-time high with attention across a variety of media sources. Slogans such as "Do not bust the crust" have become a familiar refrain in natural areas where biocrusts are common. Classroom activities linking biocrust with foundational ecological and biological concepts can help instructors develop and students experience an active learning approach, which has been shown to enhance student learning and knowledge retention (Kvam, 2000). When looking at

organisms (**Figure 2**), students can easily identify taxa (e.g., cyanobacteria, mosses, and lichens), learn about the complexities of biodiversity we cannot see, and consider diverse growth forms with differences in morphology and with similarities and differences in their function, and this information can be used to have students dissect what it means to be a species. These questions link directly to evolutionary concepts, and the close physical proximity of organisms in biocrust communities leads naturally to the topic of coexistence of species (**Figure 2**). Different types of biocrust organisms living together can be used to learn about whether the species are competing with or helping one another as they live side by side (**Figure 2**). These components can then be related to successional trajectories over time (**Figure 2**). Students can also actively manipulate the composition of biocrust communities (**Figure 2**) and, by adding water, can experience the rapid shift in activity of organisms that only moments before were dormant; or they can conduct the bare soil and biocrust fan experiment (as stated earlier) to see how soil is eroded over time learning about patterns and processes (**Figure 2**). Finally, students can tie these observations to a larger scale while sitting at a computer and looking at how much of Earth's land area (over 40+%!) is drylands where large fractions can potentially be colonized by biocrusts (Rodriguez-Caballero et al., 2018a). With that much potential to be harnessed in the communities that make up biocrusts as an educational tool, numerous questions can be asked by students, such as "how do different types of land use or different management actions change biocrusts, and how do those changes affect a patch of ground, the ecosystem, or even the world?" These are all concepts teachers can demonstrate in the classroom while providing students learning-by-doing experiences. Using biocrusts as a model system in the classroom can raise the interest in students about biological concepts and inspire individuals to enter into STEM fields and further our knowledge of the natural world.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, and further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

AF conducted the majority of the writing, with all authors providing suggestions and edits, thus enhancing the document. AA, CH, and AF created the figures and AA provided the photos used in the figures unless otherwise noted. All authors contributed to the article and approved of the submitted version.

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REFERENCES

Barger, N. N., Weber, B., Garcia-Pichel, F., Zaady, E., and Belnap, J. (2016). "Patterns and controls on nitrogen cycling of biological soil crusts" in *Biological soil crusts: An organizing principle in Drylands. Ecological studies (analysis and synthesis). Vol. 226.* eds. B. Weber, B. Büdel and J. Belnap (Cham: Springer).

Belnap, J., and Lange, O. L. (2001). *Biological soil crusts: Structure, function, and management. Ecological studies (analysis and synthesis). Vol. 150.* Berlin, Heidelberg: Springer.

Beraldi-Campesi, H., and Retallack, G. J. (2016). "Terrestrial ecosystems in the precambrian" in *Biological soil crusts: An organizing principle in Drylands. Ecological studies (analysis and synthesis). Vol. 226*. eds. B. Weber, B. Büdel and J. Belnap (Cham: Springer).

Bowker, M. A. (2007). Biological soil crust rehabilitation in theory and practice: an underexploited opportunity. *Restor. Ecol.* 15, 13–23. doi: 10.1111/j.1526-100X.2006.00185.x

Bowker, M. A., Belnap, J., Büdel, B., Sannier, S., Pietrasiak, N., Eldridge, D. J., et al. (2016). "Controls on disturbance patterns of biological soil crusts at micro to global scales" in *Biological soil crusts: An organizing principle in Drylands. Ecological studies (analysis and synthesis). Vol. 226.* eds. B. Weber, B. Büdel and J. Belnap (Cham: Springer).

Bowker, M. A., Maestre, F. T., Eldridge, D., Belnap, J., Castillo-Monroy, A., Escolar, C., et al. (2014). Biological soil crusts (biocrusts) as a model system in community, landscape and ecosystem ecology. *Biodivers. Conserv.* 23, 1619–1637. doi: 10.1007/s10531-014-0658-x

Chamizo, S., Rodriguez-Caballero, E., Roman, J. R., and Canton, Y. (2017). Effects of biocrust on soil erosion and organic carbon losses under natural rainfall. *Catena* 148, 117–125. doi: 10.1016/j.catena2016.06.017

Darby, B. J., and Neher, D. A. (2016). "Microfauna within biological soil crusts" in *Biological soil crusts: An organizing principle in Drylands. Ecological studies (analysis and synthesis). Vol. 226.* eds. B. Weber, B. Büdel and J. Belnap (Cham: Springer).

Darrouzet-Nardi, A., Reed, S. C., Grote, E. E., and Belnap, J. (2015). Observations of net soil exchange of CO2 in a dryland show experimental warming increases carbon losses in biocrust soils. *Biogeochemistry* 126, 363–378. doi: 10.1007/s10533-015-0163-7

Eldridge, D. J., Reed, S., Travers, S. K., Bowker, M. A., Maestre, F. T., Ding, J., et al. (2020). The pervasive and multifaceted influence of biocrusts on water in the world's drylands. *Glob. Chang. Biol.* 26, 6003–6014. doi: 10.1111/gcb.15232

European Commission/EACEA/Eurydice (2019). Compulsory education in Europe—2019/2020. Eurydice facts and figures. Luxembourg: Publications Office of the European Union.

Faist, A. M., Herrick, J. E., Belnap, J., Van Zee, J. W., and Barber, N. N. (2017). Biological soil crust and disturbance controls on surface hydrology in a semi-arid ecosystem. *Ecosphere* 8:eo1691. doi: 10.1002/ecs2.1691

Ferrenberg, S., Reed, S. C., and Belnap, J. (2015). Climate change and physical disturbance cause similar community shifts in biological soil crusts. *Proc. Natl. Acad. Sci. U. S. A.* 112, 12116–12121. doi: 10.1073/pnas.1509150112

Ferrenberg, S., Tucker, C. L., and Reed, S. C. (2017). Biological soil crusts: diminutive communities of potential global importance. *Front. Ecol. Environ.* 15, 160–167. doi: 10.1002/fee.1469

Kheirfam, H., Sadeghi, S. H., Darki, B. Z., and Homaee, M. (2017). Controlling rainfall-induced soil loss from small experimental plots through inoculation of bacteria and cyanobacteria. *Catena* 152, 40–46. doi: 10.1016/j.catena.2017.01.006

Kvam, P. H. (2000). The effect of active learning methods on student retention in engineering statistics. *Am. Stat.* 54, 136–140. doi: 10.1080/00031305.2000.10474526

Liu, Y., Delagado-Baquerizo, M., Trivedi, P., He, J., Wang, J., and Singh, B. K. (2017). Identity of biocrust species and microbial communities drive the response of soil multifunctionality to simulated global change. *Soil Biol. Biochem.* 107, 208–217. doi: 10.1016/j.soilbio.2016.12.003

Maestre, F. T., Bowker, M., Canton, Y., Castillo-Monroy, A. P., Cortina, J., Escolar, C., et al. (2011). Ecology and functional roles of biological soil crusts in semi-arid ecosystems of Spain. *J. Arid Environ.* 75, 1282–1291. doi: 10.1016/j.jaridenv.2010.12.008

Maestre, F. T., Bowker, M. A., Eldridge, D. J., Cortina, J., Lazaro, R., Gallardo, A., et al. (2016). "Biological soil crusts as a model system in ecology" in *Biological soil crusts: An organizing principle in Drylands. Ecological studies (analysis and synthesis). Vol. 226.* eds. B. Weber, B. Büdel and J. Belnap (Cham: Springer).

Reed, S. C., Maestre, F. T., Ochoa-Hueso, R., Kuske, C., Darrouzet-Nardi, A., Oliver, M., et al. (2016). "Biocrusts in the context of global change" in *Biological soil crusts: An organizing principle in Drylands. Ecological studies (analysis and synthesis). Vol. 226.* eds. B. Weber, B. Büdel and J. Belnap (Cham: Springer).

Rodriguez-Caballero, E., Belnap, J., Büdel, B., Crutzen, P. J., Andreae, M. O., Poschl, U. L., et al. (2018a). Dryland photoautotrophic soil surface communities endangered by global change. *Nat. Geosci.* 11, 185–189. doi: 10.1038/s41561-018-0072-1

Rodriguez-Caballero, E., Castro, A. J., Chamizo, S., Quintas-Soriano, C., Garcia-Llorente, M., Canton, Y., et al. (2018b). Ecosystem services provided by biocrusts: from ecosystem functions to social values. *J. Arid Environ.* 159, 45–53. doi: 10.1016/j.jaridenv.2017.09.005

Rodriguez-Caballero, E., Roman, J. R., Chamizo, S., Ramos, B. R., and Canton, Y. (2019). Biocrust landscape-scale spatial distribution is strongly controlled by terrain attributes: topographic thresholds for colonization in a semiarid badland system. *Earth Surf. Process. Landf.* 44, 2771–2779. doi: 10.1002/esp.4706

Samolov, E., Baumann, K., Büdel, B., Jung, P., Leinweber, P., Mikhailyuk, T., et al. (2020). Biodiversity of algae and cyanobacteria in biological soil crusts collected along a climatic gradient in Chile using an integrative approach. *Environ. Biol.* 8:1047. doi: 10.3390/microorganisms8071047

Van Goethem, M. W., Swenson, T. L., Trubl, G., Roux, S., and Northen, T. R. (2019). Characteristics of wetting-induced bacteriophage blooms in biological soil crust. *mBio* 10, e02287–e02219. doi: 10.1128/mBio.02287-19

Vitousek, P. M. (2002). Oceanic Islands as model systems for ecological studies. *J. Biogeogr.* 29, 573–582. doi: 10.1046/j.1365-2699.2002.00707.x

Weber, B., Bowker, M., Zhang, Y., and Belnap, J. (2016). "Natural recovery of biological soil crusts after disturbance" in *Biological soil crusts: An organizing principle in Drylands. Ecological studies (analysis and synthesis). Vol. 226.* eds. B. Weber, B. Büdel and J. Belnap (Cham: Springer).

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A Culturally Responsive Curricular Revision to Improve Engagement and Learning in an Undergraduate Microbiology Lab Course

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We seek to increase student engagement and success to subsequently lead to increased retention and degree attainment for students at our Hispanic-serving institution. We hypothesized that using a culturally responsive approach in an undergraduate microbiology lab would increase engagement and learning gains. Using a culturally responsive approach allowed students to start their learning from their own place of understanding—centering students' lived experiences. Students interviewed family members to learn about "home remedies," and then devised experiments to test whether those home remedies affected growth of bacteria commonly implicated in gastrointestinal distress (*Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli*) or sore throat (*Neisseria gonorrhoeae*, *Streptococcus pyogenes*, and *Mycoplasma pneumoniae*). As a final assessment, students generated project posters which they presented at a class symposium. Implementation of a culturally responsive research experience focused on the gut microbiome resulted in increased learning gains as evidenced by movement up Bloom's Revised Taxonomy Scale. Student feedback indicated increased engagement, increased confidence in communicating science and a deeper understanding and appreciation for microbiology. Taken together, the results indicate that students appreciate a more culturally responsive and student-centered approach to learning in microbiology and encourages expansion of this approach to other modules in the course. This paper includes responsive data to support this claim, as well as a sample course calendar and supplementary learning material to support the human microbiome approach to microbiology.

Keywords: curriculum, undergraduate, research, culturally responsive, pedagogy

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INTRODUCTION

Bacterial metabolism is a core concept for undergraduate microbiology education (Merkel and Reynolds, 2014). The complexity of this multi-dimensional topic can make it difficult for students to grasp core concepts in a meaningful way as they struggle to find the significance of these concepts relative to their lived experiences. On the other hand, antibiotic sensitivity has immediate

"real-life" applications and students easily grasp the concept. Yet, the common ways to teach antibiotic sensitivity—through antibiotic disc or similar cookbook-style zone of inhibition lab experiments—can seem simplistic.

In our course assessments, students consistently demonstrate low engagement with the bacterial metabolism module and struggle to relate the concepts of bacterial metabolism to larger themes, such as the human health and physiology. Generally, students rate our antibiotic sensitivity module, low on the engagement scale, even as they meet the learning goals associated with the module. We sought to improve engagement and learning in these important themes in our undergraduate microbiology course by combining them under the overall topic of the human microbiome.

The human microbiome is a key influencer of our mental and physical health (Shreiner et al., 2015; Malan-Muller et al., 2017; Fitzgibbon and Mills, 2020). Recent studies test long-held claims that certain dietary plants contribute to health (Katoch et al., 2013; Praditya et al., 2019). As scientists learn more about the interactions between the food people consume and the microbiome, the role of these interactions in influencing a wide range of diseases, including obesity, allergies, metabolic diseases and cancer, becomes more evident (Gensollen et al., 2016). In the future, microbiome evaluation may become an important part of disease diagnosis and treatment. Therefore, it is important that emerging scientists begin to grasp these concepts now to understand the role of microbiome in maintaining health as medical science moves toward a more personalized approach to treatments.

As such, we sought to revise our microbiology curriculum to include microbiome-specific learning experiences. We merged two course modules (bacterial metabolism and antibiotic sensitivity) to create one curricular undergraduate research experience (CURE) in the accompanying lab course. We selected these modules because bacterial metabolism typically has low learning goals success rate and antibiotic sensitivity typically has low engagement rankings.

Several studies have described increased problem-solving abilities and critical thinking, when CUREs are implemented in the undergraduate biology classroom (Cooper et al., 2019; Hurst-Kennedy et al., 2020). The 2012 *Engage to Excel* report from the President's Council of Advisors on Science and Technology encouraged discovery-based research experiences as part of STEM curriculum to increase student engagement and ownership of projects (Olson and Riordan, 2012). To ensure the CURE met the course learning outcomes, we adapted a backward design framework to build the CURE into the curriculum (Reynolds and Kearns, 2017). Our intervention seeks to increase learning gains and engagement through relevancy to improve the student learning experience.

To make the content more responsive, we incorporated culturally responsive teaching (CRT) into the CURE design process (Aronson and Laughter, 2016; Cooper et al., 2017; Gay, 2018). In particular, the teaching goals are discreetly outlined to use cultural knowledge as context for the research project (**Table 1**). The initial background knowledge for the CURE is gathered using ethnographic interviews of family members. Using techniques of ethnographic research ground the researcher's approach in cultural knowledge and awareness and place social knowledge in parallel prominence with scientific knowledge (Atkinson and Pugsley, 2005). This approach, using documented family knowledge for direct engagement in a microbiome research question, has been described previously with success in increased engagement by Pérez-Losada et al. (2020). In their study, students compared skin microbiome composition using a "grandma hypothesis"—areas of the body that grandmothers are always telling their grandchildren to clean carefully, such as behind the ears and between the toes.

The CRT framework increases access to the skills, knowledge, and competencies necessary for success (Aronson and Laughter, 2016). This is a high priority in our upper-division biology courses, as success in these courses correlates to post-graduation transfer and persistence in science programs at 4-year colleges (Woods, n.d.). Culturally responsive teaching embraces cultural knowledge, personal frames of reference, and lived experiences to make learning more responsive to and effective for students (Snively and Corsiglia, 2001; Hernandez et al., 2013).

Research studies have also described a retention and belongingness benefit to undergraduate CUREs for students from underrepresented groups (Bangera and Brownell, 2014; Elgin et al., 2016). As the majority of our students identify as Black and/or Latino, both CRT and CURE promised benefits uniquely suited to the success of our student population.

In a previous study, Skendzic and Keler (2019) describe a fruit fly microbiome CURE in which students supplemented fruit fly media with various antibiotics, probiotics, and common food additives (i.e., monosodium glutamate and food dye), then assessed the effect of these supplements on the growth of *Lactobacillus*, the most prevalent bacteria in the fruit fly gut. We have extended this type of inquiry-based experiment by adding a personal component.

In the CURE we describe in this paper, students used principles of ethnographic research to engage in family interviews to determine which variables (home remedies) to include in their research. This data was paired with what they have learned in the antibiotic resistance module to set up a comparative study. Thus, in addition to studying the effects of purported antibacterial substances on common bacterial populations, students could make direct connections between the microbiome and overall health.

The inclusion of ethnographic research as the foundation of a series of student-derived experiments allows students to demonstrate their understanding of bacterial metabolism and antibiotic sensitivity while demonstrating mastery of bacterial culture techniques in a project with a personal connection. We hypothesized that a CRT-CURE intervention would increase engagement with these topics by making them more responsive to students and this increased relevancy would promote learning gains. Furthermore, we speculated increased engagement in the course might alleviate some of the ostracizing learning experiences underrepresented students often face in science classes by centering the context of the material around their own communities and cultural practices. This study builds on the success of other well-known CURES, such as the Bean

TABLE 1 | Example of mapping one outcome according to CURE Backward design process with embedded culturally responsive teaching (CRT) strategies.

Backward design step	Scientific discovery	CRT practice
Identify desired outcomes	Students determine if selected foods have antimicrobial properties	• Affirm that cultural traditions have ways of knowing. • Cross-cultural exchange from student-student
Determine acceptable evidence	Students measure zone of inhibition from food samples, using antibiotics as controls	• Student as knowledge holder—professor as facilitator • Scaffolded assignment with clear expectations at each step
Planned learning experiences and instruction	(1) Interview family knowledge holders (2) Culture bacteria (3) Measure CFU from serial dilution (4) Design and implement zone of inhibition study to test food item(s)	• Learning within the context of culture • Students research aspects of the topic in their community • Students share their work with their family/community using culturally appropriate language

Using the backward design process ensured that the introductory labs provided sufficient training and knowledge for student independence during the research project. THE CRT practice principles were visible in each outcome of the research project.

Beetle Microbiome CURE, by adding a personal component to an authentic research experience (Zelaya et al., 2020). Having students combine cultural knowledge with microbiology theory to create a novel research question adds value to the educational process and traditional practices and can begin to dismantle traditional modes of formal education, engaging a diverse student body (Baines and Zarger, 2017).

MATERIALS AND METHODS

The Assignment

Students learned typical microbiology lab techniques during the first 6-weeks of the lab (hereafter referred to as "introductory labs"). At the end of each introductory lab module, students completed a two-question engagement survey (**Table 2**). For homework, students completed Thought Questions related to the lab on an adaptive learning platform (**Table 3**). The questions were scaled according to Bloom's Revised Taxonomy Scale, with the first question from the remembering domain and the subsequent questions moving up the scale through the understanding, applying, and analyzing domains (Krathwohl, 2010).

Only if students answered a question correctly would they see the next question in that series. If students answered a question incorrectly, the session would end automatically. Once students have learned the necessary techniques and background knowledge through the introductory labs, they embark on a carefully scaffolded research project that has four main components: (1) interview, (2) library database research, (3) lab research, and (4) culminating research report. Standard biosecurity and institutional safety procedures were adhered to during this lab course.

The CURE began in Week 7 with a simple question: If you had a stomachache or a sore throat, who in your family would you ask for a remedy? Students interview a family member about a home remedy for an upset stomach and/or a sore throat, then use the ethnographic interview to generate a set of observations. Interviewing family members about health practices provided the real-world context that is important for making long-lasting curricular connections. Students recorded their interviews and organized them into a set of observations they could use as the basis for research to find preliminary scientific data to support the claims of the knowledge-holder. For instance, a grandmother describes a baking soda-honey solution she uses to soothe a sore throat, which prompts a student to search library databases to find sources related to the antimicrobial properties of sodium bicarbonate and honey.

After students completed their interviews, we used class discussions to generate common research questions. The class agreed on the following research questions: (1) Does the home remedy kill bacteria, or does it stop the symptoms? and, (2) How does the home remedy compare to antibiotic treatment? Similarly, we agreed on common class hypotheses: H_1—The food additive inhibits bacterial growth; H_0—The food additive does not inhibit growth. Interestingly, in class discussion, students agreed that even if the food additive does not inhibit growth, it may be effective in treating the symptoms. Bacteria commonly implicated in gastrointestinal distress (*Staphylococcus aureus, Bacillus cereus*, and *Escherichia coli*) or sore throat (*Neisseria gonorrhoeae, Streptococcus pyogenes*, and *Mycoplasma pneumoniae*) were used in the study.

As a final step in the pre-planning process, students generated individual hypotheses for their experiments, which were validated by peer-review (Ex. I hypothesize that chamomile tea reduces bacterial growth). Then students designed an experiment to test their hypotheses. Before starting experimentation, students met individually with the instructor for experimental design approval. Afterward, students were free to conduct their experiments at their own pace, within the allotted 4-week time period. To manage a course with 23 concurrent independent experiments, we created a lab schedule for certain tests. For instance, students would plan to have their experiment complete and a sample set ready for gram staining during Week 9 when the supplies and materials for gram staining were available in the lab (**Table 4**). After the weekly experimentation was done, students had a second attempt at the previous Thought Questions on the same adaptative learning platform. To conclude the research experience, students created research posters to present their projects to the campus community (**Supplementary Figure 1**). Thus, in our experimental design, the introductory labs served as the control group and the CURE intervention served as the experimental group.

It is also important to mention as part of the assignment, students gave consent for the instructor to submit on their behalf their research project abstracts for conference presentations. Two

TABLE 2 | After each introductory and CURE lab, students completed the engagement survey below anonymously.

	1—Strongly disagree	2—Disagree	3—Neither agree nor disagree	4—Agree	5—Strongly agree
This lab was interesting					
I used my own creativity during this lab.					

TABLE 3 | Post-lab Thought Questions were deployed using adaptive learning software after each introductory and CURE lab.

Lab topic	Domain: remembering	Domain: understanding	Domain: applying	Domain: analyzing
Lab 1—Bacterial Staining; Identify bacteria by morphology	1. Label the parts of the bacterial cell on the drawing below. 2. Create a chart to organize the staining techniques and the types of bacteria they stain, including predicted color results*.	3. Classify the bacteria below according to morphology and. 4. Classify the bacteria according to stain.	5. A spore has a mutation that prevents the production of keratin in the membrane, describe how this would affect the results of the spore stain test.	
Lab 2—Aseptic Technique—streak plates, inoculate liquid cultures	1. Predict the results from your Kirby-Bauer experiment.	2. Make a comparison chart of the types of selective media used in today's experiment. 3. Based on your experimental data, which antibiotic is most effective for your given bacterial sample.	4. Which media would you use to culture [a given bacteria]?	5. Using the class data, which antibiotic is most effective for many types of bacteria? Which has the least effectivity?
Lab 3—Serial dilutions; antibiotic sensitivity and the Kirby-Bauer test		1. Explain why serial dilutions are important for bacterial cultures.	2. Given a sample with known concentration X, design a serial dilution to produce final concentration Y.	3. Use the given set of results to match the bacterial plates with the serial dilution tube.
Lab 4—Identify bacteria by biochemistry	1. Students fill in a chart including names of test, brief description of test, examples of bacteria that would test positive*.	2. Given a data set, students can identify the type of bacteria used in the test.	3. Plan and conduct an experiment using the test you have been assigned.	4. Using class data, identify the unknown bacteria.
Lab 5—Bacterial metabolism/cellular respiration	1. Compare and contrast fermentation and cellular respiration	2. When would a metabolically versatile microbe perform fermentation rather than cellular respiration?	3. Can protein catabolism help identify microbes?	

*Each question series begins with a question from the Remembering domain. If students answered a question incorrectly, they were automatically exited from the question series. *Question not included in CURE lab and omitted from statistical analysis.*

TABLE 4 | CRT-CURE lab course calendar with associated tasks.

Week	Lab topic	Research project task
1	Identify bacteria by morphology Bacterial staining lab (gram and acid-fast stain)	Project Introduction and overview (no tasks)
2	Aseptic technique Kirby-Bauer test	Ethnographic interviews
3	Serial dilutions and growth curves	Class data gathering
4	Biochemical testing to identify bacteria	Information Literacy (library research)
5	Cellular respiration and fermentation	Research Question brainstorm
6	Research project	Hypothesis forming
7		Research plan review and approval
8		Serial dilutions and growth curves Kirby-Bauer test
9		Gram-staining Biochemical testing
10		Cellular respiration and fermentation test
11		Research report writing time
12		Class symposium

Weeks 1–5 are introductory skills-building labs. In weeks 8–10, students use the skills they learned in the introductory modules for their own experimentation.

projects were accepted for the student e-poster competition at the 2019 American Association for the Advancement of Science (AAAS) Annual Meeting.

Survey Methods

Student engagement surveys were conducted anonymously and collected on a single tablet via Google Forms at two points in the semester—immediately after each introductory lab and during the CURE. The introductory labs were traditional guided inquiry labs that focused on skill-building (how to use the tools, do the technique appropriately, and analyze the type of data generated by the technique accurately). Students responded to two prompts: "This lab was interesting," and "I used my own creativity during this lab" using a Likert scale where 1 = strongly disagree, 2 = disagree, 3 = neither agree nor disagree, 4 = agree and 5 = strongly agree. *Strongly agree* and *agree* categories were aggregated to indicate a positive engagement response. *Strongly disagree* and *disagree* categories were aggregated to indicate a negative engagement response.

For the Thought Questions, pre- and post-intervention grades were paired, then anonymized by a research assistant and analyzed after the course was over. De-identified open-ended comments were collected through student evaluations at the end of the course.

Data Analysis Methods

De-identified, raw data was provided by Dr. Fuller in a Microsoft Excel spreadsheet. The data was comprised of two, parallel satisfaction items that were measured using a five-point Likert scale. The five response categories for the Likert scale were; 1-strongly disagree, 2- disagree, 3- neither agree nor disagree, 4, agree, 5 strongly agree. The pre-condition (SatisReg) satisfaction responses ($n = 234$) were collected over the first five lab sessions. Post-condition (SatisCURE) responses were collected for the five lab sessions ($n = 240$) following the intervention.

Using SPSS, the Likert scaled data was entered and coded according to the point values of each response category. Four new variables (i.e., SatisRegAlla, SatisRegAllb, SatisCUREAlla, SatisCUREAllb) were created by calculating the sum of all the Likert scores for cases with a full set of responses ($n = 21$). Numerical and graphical assumption tests were performed on the newly transformed variables, and all assumptions for normality were met. The means of the variables were analyzed using right, one-tailed, paired *t*-tests ($\alpha = 0.05$) to determine if there was a statistically significant increase between the means of the pre- and post-groups for each of the two questions.

De-identified raw data from the Thought Questions was collected and coded as *correct* vs. *incorrect* and *did not attempt* vs. *attempt*. In addition to the raw scores, Thought Question scores were categorized according to the first four levels of Bloom's Revised Taxonomy scale (i.e., Remembering, understanding, applying, and analyzing). Using SPSS, the data was entered and coded. Assumptions for normality were met by the Central Limit Theorem. Two-proportion *t*-tests ($\alpha = 0.05$) were performed comparing introductory lab data and CURE data for the same Thought Questions. We then analyzed the number of correct responses and question attempts along the Bloom's Revised scale after the CURE intervention.

RESULTS

We used student interest to measure engagement. Student interest in lab work increased during the CURE, compared to their interest in the introductory labs. Students also reported the CURE lab structure allowed them to be more creative in their experiments (**Table 5**).

To measure learning gains, at the end of each introductory module, students were given a series of questions that moved progressively up the first four levels of Bloom's scale: (1) remembering, (2) understanding, (3) applying, and (4) analyzing. Students were given the same set of questions after each related experiment during the CURE. More students attempted questions in the higher domains (understanding, applying, and analyzing), suggesting that students were more confident to try the more difficult questions after doing the experiments in the context of the CURE (**Figure 1**). Overall, students answered more Thought Questions correctly during the CURE than during the introductory labs (**Figure 2**).

During the end-of-semester student evaluations, we asked students to tell us if the overall course experience changed their views of themselves as scientists. Wherein, we anticipated students to mention a growth in skills and ability, many comments addressed a deeper appreciation for the nature of science and what it means to be a researcher (**Table 6**).

"This lab was interesting."

The mean of the post-condition group ($\bar{x} = 21.9$; $SD = 1.7$) was greater than the mean of the pre-condition group ($\bar{x} = 16.9$; $SD = 2.3$). There was a statistically significant increase in students' perceived interest in the labs from the pre-condition group to the post-condition group [$t(20) = 7.3$, $p < 0.0001$, right one-tailed, *t*-test]. Results show that there is enough evidence to reject the null hypothesis of no difference.

"I used my own creativity during this lab."

The mean of the post-condition group ($\bar{x} = 19.8$; $SD = 1.4$) was greater than the mean of the pre-condition group ($\bar{x} = 11.0$; $SD = 1.9$). There was a statistically significant increase in students' perceived use of their own creativity in the labs from the pre-condition group to the post-condition group [$t(20) = 16.54$, $p < 0.0001$, right one-tailed *t*-test]. Results show that there is enough evidence to reject the null hypothesis of no difference.

DISCUSSION

This study had two pedagogical goals—to increase student engagement and to increase student learning outcomes. We addressed these two issues by focusing on using an inquiry-based microbiome study driven by student-generated questions. An ideal laboratory project allows students to: (1) apply theory to practice, (2) use critical thinking to apply the scientific process, and (3) increase interest and motivation (Nicolaidou et al., 2019).

Success with culturally responsive pedagogies has been well-documented in K-12 education. However, there is a lack of

TABLE 5 | Assessment of student engagement.

	Introductory Labs	CURE labs
This lab was interesting (strongly agree/agree)	16.9	21.9[a]
I used my own creativity during this lab (strongly agree/agree)	11	19.8[b]

Student engagement in the introductory and CURE labs was measured by anonymous survey responses to the prompts to gauge student interest and creativity. Mean scores for strongly agree/agree were aggregated and compared between introductory and CURE post-lab surveys.
[a]*SD = 1.7, p < 0.0001.*
[b]*SD = 1.4, p < 0.0001.*

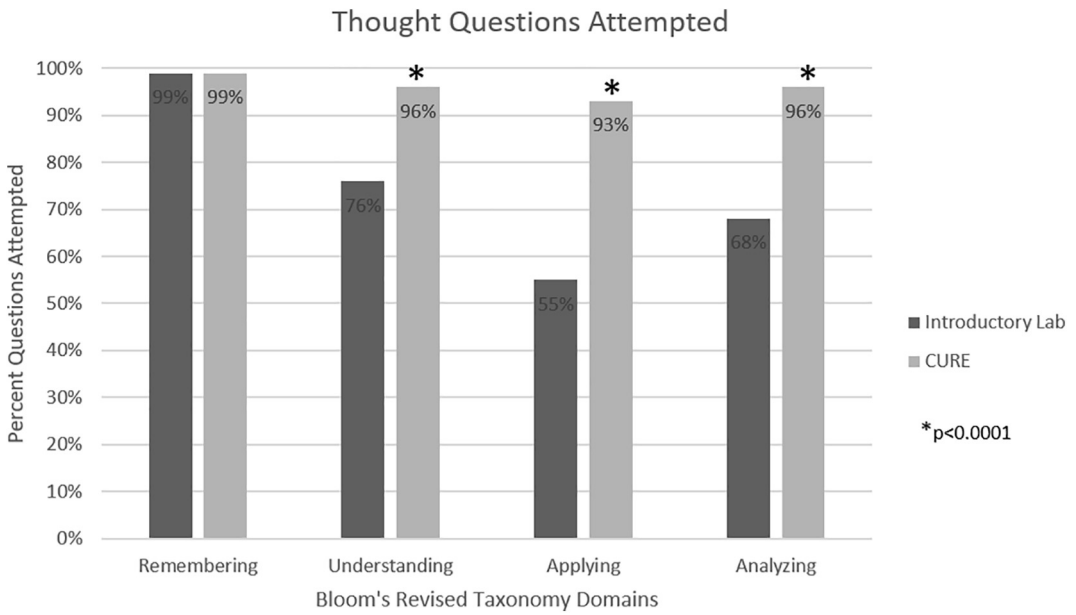

FIGURE 1 | Attempted responses. Comparison of the percent of students who attempted Thought Question at each domain of the Bloom's Revised Taxonomy scale (remembering) after the introductory and CURE labs. *$p < 0.0001$.

published studies outlining the success and implementation guidance on CRT at the undergraduate level, particularly in the sciences.

On the other hand, there is much data to support CUREs as a beneficial intervention in undergraduate biology with reports documenting improvements in learning, belongingness, and retention (Rainey et al., 2018; Nerio et al., 2019). Here we describe a culturally responsive CURE that combines the engagement benefits of CRT and the learning benefits of CURE as a successful model for teaching and learning in an undergraduate microbiology course.

In addition to the learning gains, or perhaps in support of them, this course re-design gave more opportunities for formative assessment because of the close scaffolding of the research project, one-on-one meetings for research design approval, and pre- and post-proposal student/student peer-review. In the end, students were able to relate the concept of the microbiome to their personal experiences by learning about human gut bacteria commonly implicated in illnesses and how bacterial populations are affected by the foods we eat.

Asking students to design experiments based on family stories is quite a shift in the way we normally teach microbiology. In doing so, we had to step outside of the normal formula of "teach, show, do" to evolve the learning experience by incorporating a deeper exploration of topics in a skills-based environment across the entire semester. Introducing students to theory in lecture, accompanied by demonstrations and training in lab are common for undergraduate science courses and can certainly get students to meet the course learning outcomes. However, stretching the student experience by deepening learning through student-generated research has additional benefits. The use of ethnographic interviews to gather initial observations allows a constructivist approach to teaching. This shifts the instructor-student relationship from one of knowledge holder (instructor) and knowledge receiver (student) to one that places students as active knowledge constructors and allows the instructor to act as facilitator (Lee and Hannafin, 2016). Thus, the aim of this project is to re-center the learning experience around the student by breaking away from the cookbook model of laboratory science and facilitating an outward learning process that has the students' lived experience at the core in an effort to strengthen engagement.

Due to the success of this intervention, we are currently re-designing two additional courses (one statistics course and one introduction to biology course) to include a CRT-CURE

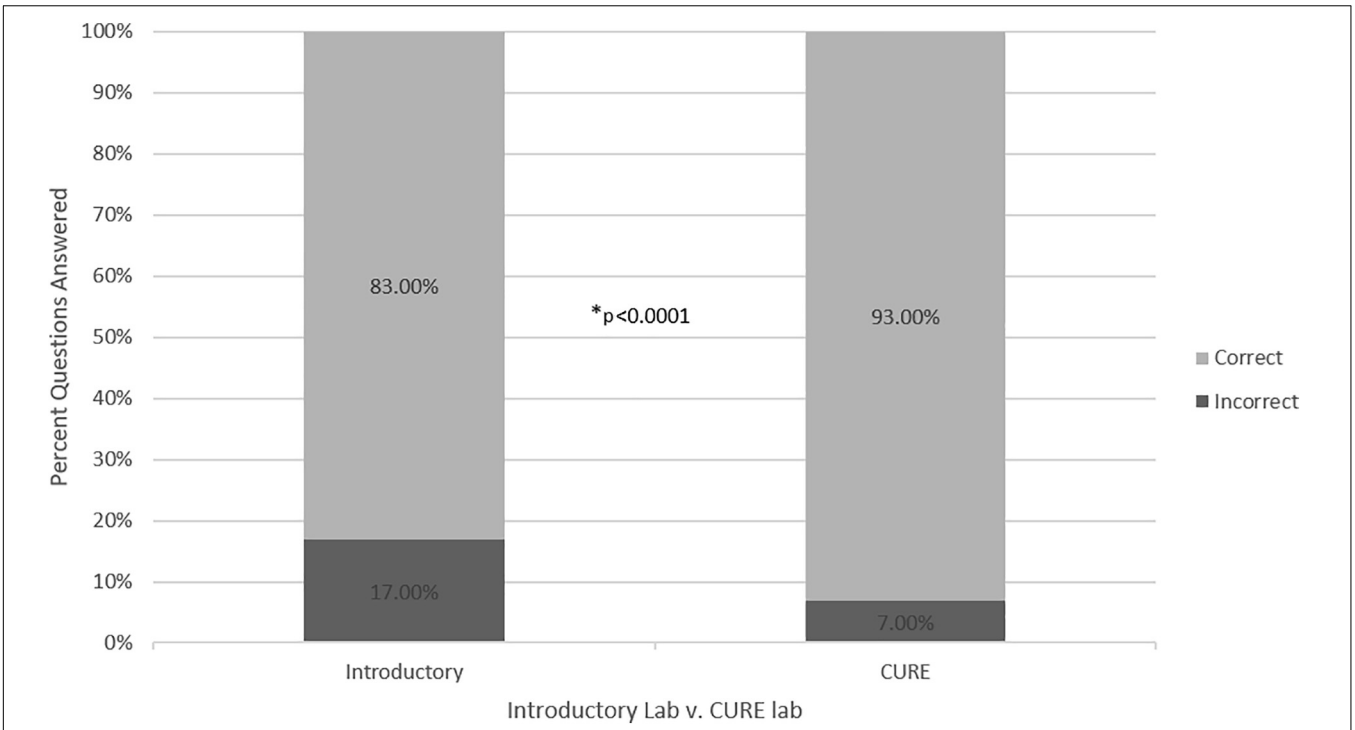

FIGURE 2 | Correct answers. Comparison of the proportion of correct answers per each Thought Question attempt after the introductory and CURE labs. *$p < 0.0001$.

TABLE 6 | Student feedback from open-ended question: "Has the research project changed your perceptions of doing science?"

Before I used to get mad when my experiments didn't work. But because we had more time to work through our problems during the research project, I feel like I learned from my mistakes.
I feel like my family respects my career goals more. When I talked to them about my project, they were interested and impressed that I was using microbiology to test my grandmother's remedies.
I feel like I have a better understanding of how research fits into like general medical care. There is a lot of work that would go into creating one medical treatment.
[The instructor] is always calling us scientists and specialists, but this time I really felt like I was a scientist!
Working on my own research helped me grow as a scientist.
Even though I was nervous to do the project on my own, I felt more confident because we could use each other as collaborators. For once we were talking about our research when we met in the commons instead of just chatting about nothing.
I never thought I could design a project, do the research, then create a good poster. This is also the first time that I was asking questions during presentations because I really wanted to know the answers not just to get the participation grade.
I had a really hard time getting my experiments to work but [the instructor] kept encouraging me and in the end I figured it out. I don't think I'll ever do scientific research as a career, but I do feel more confident for the next classes because I know if I take my time and follow my mistakes I can figure it out.
Even though we worked on individual projects, I really felt like we were a team. We had to peer-review each other's papers and [the instructor] made us ask each other for help before we asked her so everybody was really helping each other.
For once I see that the things I'm learning in my classes will actually help me when I get a career. Like, I can really do these experiments if I got this kind of job. And I have a whole project in my ePortfolio that proves it.
I never really had a connection with the research projects and experiments in my classes. I want to be a doctor so I can help my community but this project has me thinking about ways that different types of scientists can help communities. I never thought about it like that before.

intervention. We intend to continue strategic course re-designs so that each student in the science program will have at least two CRT-CURE courses as they matriculate through the Associate's Degree program.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

This study involving human participants was reviewed and approved by the City University of New York Institutional Review Board (IRB# 2020-0324). Student participants gave written informed consent prior to this study.

AUTHOR CONTRIBUTIONS

KF conceived of and implemented the study and wrote the manuscript. CT carried out the data analysis for the manuscript. Both authors contributed to the article and approved the submitted version.

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We thank to our students and their families for sharing their stories and home remedies.

BIOSAFETY

This project was performed in a Biosafety Level 1 laboratory following all of the required safety measures for handling and disposal of biological agents using non-infectious strains of the aforementioned biology.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.577852/full#supplementary-material

Supplementary Figure 1 | Sample student poster.

REFERENCES

Aronson, B., and Laughter, J. (2016). The Theory and Practice of Culturally Relevant Education: A Synthesis of Research Across Content Areas. *Review of Educational Research* 86, 163–206. doi: 10.3102/0034654315582066

Atkinson, P., and Pugsley, L. (2005). Making Sense of Ethnography and Medical Education. *Medical Education*, no. 39, 228–234. doi: 10.1111/j.1365-2929.2004.02070.x

Baines, K., and Zarger, R. K. (2017). 'It's Good to Learn about the Plants': Promoting Social Justice and Community Health through the Development of a Maya Environmental and Cultural Heritage Curriculum in Southern Belize. *Journal of Environmental Studies and Sciences* 7, 416–424. doi: 10.1007/s13412-016-0416-3

Bangera, G., and Brownell, S. E. (2014). Course-Based Undergraduate Research Experiences Can Make Scientific Research More Inclusive | CBE—Life Sciences Education. *CBE - Life SCiences Education* 13, 29–40.

Cooper, K. M., Blattman, J. N., Hendrix, T., and Brownell, S. E. (2019). The Impact of Broadly Relevant Novel Discoveries on Student Project Ownership in a Traditional Lab Course Turned CURE. *CBE—Life Sciences Education* 18, ar57. doi: 10.1187/cbe.19-06-0113

Cooper, K. M., Soneral, P. A. G., and Brownelli, S. E. (2017). Define Your Goals before You Design a CURE: A Call to Use Backward Design in Planning Course-Based Undergraduate Research Experiences. *Journal of Microbiology & Biology Education* 18, 1–7.

Elgin, S. C. R., Bangera, G., Decatur, S. M., Dolan, E. L., Guertin, L., Newstetter, W. C., et al. (2016). Insights from a Convocation: Integrating Discovery-Based Research into the Undergraduate Curriculum. *CBE—Life Sciences Education* 15, fe2. doi: 10.1187/cbe.16-03-0118

Fitzgibbon, G., and Mills, K. H. G. (2020). The Microbiota and Immune-Mediated Diseases: Opportunities for Therapeutic Intervention. *European Journal of Immunology* 50, 326–337. doi: 10.1002/eji.201948322

Gay, G. (2018). *Culturally Responsive Teaching: Theory, Research, and Practice*. Electronic resource. Third edition. Multicultural Education Series. New York, NY: Teachers College Press.

Gensollen, T., Iyer, S. S., Kasper, D. L., and Blumberg, R. S. (2016). How Colonization by Microbiota in Early Life Shapes the Immune System | Science. *Science* 352, 539–544. doi: 10.1126/science.aad9378

Hernandez, C. M., Morales, A. R., and Shroyer, M. G. (2013). The Development of a Model of Culturally Responsive Science and Mathematics Teaching. *Cultural Studies of Science Education* 8, 803–820. doi: 10.1007/s11422-013-9544-1

Hurst-Kennedy, J., Saum, M., Achat-Mendes, C., D'Costa, A., Javazon, E., Katzman, S., et al. (2020). The Impact of a Semester-Long, Cell Culture and Fluorescence Microscopy CURE on Learning and Attitudes in an Underrepresented STEM Student Population. *Journal of Microbiology & Biology Education* 21, 21.1.25. doi: 10.1128/jmbe.v21i1.2001 **21.1.25,

Katoch, A., Batta, B., Kumar, A., and Sharma, P. C. (2013). Screening of *Murraya koenigii* (Curry) and *Camellia sinensis* (Tea) leaves for antimicrobial activity against strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida* species and their phytochemical analysis. *Intern. J. Pharm. Sci. Res.* 4:862.

Krathwohl, D. R. (2010). A Revision of Bloom's Taxonomy: An Overview. *Theory Into Practice* 41, 212–218. doi: 10.1207/s15430421tip4104_2

Lee, E., and Hannafin, M. (2016). A Design Framework for Enhancing Engagement in Student-Centered Learning: Own It, Learn It, and Share It. *Educational Technology Research & Development* 64, 707–734. doi: 10.1007/s11423-015-9422-5

Malan-Muller, S., Valles-Colomer, M., Raes, J., Lowry, C. A., Seedat, S., and Hemmings, S. M. J. (2017). The Gut Microbiome and Mental Health: Implications for Anxiety- and Trauma-Related Disorders. *OMICS: A Journal of Integrative Biology* 22, 90–107. doi: 10.1089/omi.2017.0077

Merkel, S., and Reynolds, J. (2014). *Recommended Curriculum Guidelines for Undergraduate Microbiology Education." ASM Task Force for Curriculum Guidelines*. **Q.

Nerio, R., Webber, A., MacLachlan, E., Lopatto, D., and Caplan, A. J. (2019). One-year research experience for associate's degree students impacts graduation, STEM retention, and transfer patterns. *CBE Life Sci. Educ.* 18:ar25. doi: 10.1187/cbe.19-02-0042

Nicolaidou, V., Nicolaou, P., and Nicolaou, S. (2019). Transforming a Cookbook Undergraduate Microbiology Laboratory to Inquiry Based Using a Semester-Long PBL Case Study. *Advances in Physiological Education* 43, 82–92. doi: 10.1152/advan.00167.2018

Olson, S., and Riordan, D. G. (2012). *Engage to Excel: Producing One Million Additional College Graduates with Degrees in Science, Technology, Engineering, and Mathematics. Report to the President. Executive Office of the President*. Executive Office of the President. Available online at: https://eric.ed.gov/?id=ED541511**Q.

Pérez-Losada, M., Crandall, K. M., and Crandall, K. A. (2020). Testing the 'grandma hypothesis': characterizing skin microbiome diversity as a project-based learning approach to genomics. *J. Microbiol. Biol. Educ.* 21:21.1.7. doi: 10.1128/jmbe.v21i1.2019

Praditya, D., Kirchhoff, L., Brüning, J., Rachmawati, H., Steinmann, J., and Steinmann, E. (2019). Anti-infective properties of the golden spice curcumin. *Front. Microbiol.* 10:912. doi: 10.3389/fmicb.2019.00912

Rainey, K., Dancy, M., Mickelson, R., Stearns, E., and Moller, S. (2018). Race and gender differences in how sense of belonging influences decisions to major in STEM. *Intern. J. STEM Educ.* 5:10. doi: 10.1186/s40594-018-0115-6

Reynolds, H. L., and Kearns, K. D. (2017). A Planning Tool for Incorporating Backward Design, Active Learning, and Authentic Assessment in the College Classroom. *College Teaching* 65, 17–27. doi: 10.1080/87567555.2016.1222575

Shreiner, A. B., Kao, J. Y., and Young, V. B. (2015). The Gut Microbiome in Health and in Disease. *Current Opinion in Gastroenterology* 31, 69–75. doi: 10.1097/MOG.0000000000000139

Skendzic, E., and Keler, C. (2019). Fruit flies & the gut microbiome: redesign your-bacteria lab exercise. *Am. Biol. Teach.* 81, 47–51. doi: 10.1525/abt.2019.81.1.47

Snively, G., and Corsiglia, J. (2001). Discovering indigenous science: implications for science education. *Science Education* 85, 6–34.

Woods, K. L. (n.d.). *Community College Transfer Student Success: The Role of Community College Academic Experiences on Post-Transfer Adjustment," 217*.

Zelaya, A., Gerardo, N., Blumer, L., and Christopher, B. (2020). The bean beetle microbiome project: a course-based undergraduate research experience in microbiology. *Front. Microbiol.*

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Students' Experiences of Working With a Socio-Scientific Issues-Based Curriculum Unit Using Role-Playing to Negotiate Antibiotic Resistance

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The emergence and widespread of antibiotic-resistant pathogenic microorganisms are of great individual and societal relevance. Due to the complex and multilayered nature of the topic, antibiotic resistance (ABR) is the object of concern for several scientific fields, such as microbiology or medicine, and encompasses a broad range of political, economic, and social aspects. Thus, the issue related to antibiotic-resistant bacterial diseases offers an excellent platform for designing and implementing the teaching and learning of socio-scientific issues (SSI). We created a SSI-based curriculum unit for use in secondary science classrooms by developing a collaborative partnership between education researchers and microbiologists. This classroom environment allows students to explore and negotiate ABR as a societal and scientific phenomenon. For this purpose, we leveraged role-playing within the SSI-based unit as a productive context for engaging students in learning opportunities that provide multiple perspectives on ABR and the complex interplay of its accelerators. This case-based paper describes Austrian school students' experiences from their participation in a SSI-embedded role-playing classroom environment and subsequent activities that included a mini congress with a poster presentation and a panel discussion. An open-ended questionnaire-based assessment tool was used to examine the situational characteristics of the students' work. To assess students' contributions, we applied a qualitative content analysis design and identified cognitive and affective outcomes. The students' learning experiences demonstrate that they considered the content – the social complexities of antibiotic-resistant bacteria and associated diseases – exciting and very topical. The students perceived that learning about ABR is relevant for their future and involves both individual and societal responsibility for action. Although the curriculum unit and its assignments were described as labor-intensive, it became apparent that the role-playing setting has the potential to inform students about multiple stakeholder positions concerning ABR. Concerning the promotion of science practices, almost all students claimed that they learned to organize, analyze, evaluate, and present relevant information. Moreover, the students affirmed that they learned to argue from the perspective of their assigned roles. However, the students did not clarify whether they learned more through this SSI-based classroom instruction than through conventional science teaching approaches.

Keywords: socio-scientific issues, educational strategies, students' experiences, science and society, antibiotic resistance, science communication

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INTRODUCTION

Science education and practice aim to support society in acquiring skills that enable citizens to make well-informed decisions and form evidence-based opinions on current societal challenges (Dawson and Venville, 2010; Osborne, 2010). Antimicrobial resistance (AMR) is a pressing and capacious problem in the field of Science|Environment|Health pedagogy (Zeyer and Dillon, 2019). In the coming years, this significant public health issue will progressively gain importance for both public discourse as well as science teaching because of its potential to affect humankind's personal, social, and global patterns of behavior (Fensham, 2012).

As the primary form of AMR, antibiotic resistance (ABR) occurs when bacteria adapt and increasingly acquire resistance to antibiotic agents to which they were formerly susceptible (Depardieu et al., 2007; Davies and Davies, 2010; Blair et al., 2015). Usually, ABR and AMR, respectively, are a consequence of natural adaptive selection by a genetic mutation (Andersson and Hughes, 2010). It allows bacteria, particularly those frequently found in healthcare settings, to resist the noxious effects of specific antibiotic agents (Alekshun and Levy, 2007). Although numerous previously deadly infectious diseases have turned into non-life-threatening inconveniences in the antibiotic era, this outstanding scientific progress is unfortunately jeopardized (Carlet et al., 2012; Ventola, 2015).

Infections caused by antibiotic-resistant pathogens have become a growing threat to modern public health care that requires action across all economic and societal sectors, from individuals to communities and from hospitals to entire healthcare systems (Carlet et al., 2011; Laxminarayan et al., 2013). As the resistant pathogens might persist in human or animal organisms, ABR endangers the effective management, prevention, and medical procedure of an ever-increasing range of healthcare-associated infectious diseases. Previous research conservatively estimated that AMR is responsible for 700,000 deaths per year globally (O'Neill, 2016). In a recent study, Cassini et al. (2019) reported that more than 33,000 deaths, which were assignable to infections with selected antibiotic-resistant bacteria, occurred in countries of the European Union and the European Economic Area in 2015.

Human activities, such as misuse and overuse of antibiotics, inadequate hygiene precautions, and unfavorable practices in healthcare settings or the food chain, have accelerated the emergence and transmission of drug-resistant pathogens (Carlet et al., 2011). Apart from their every-day usage for clinical purposes in human and veterinary medicine, the misuse of antibiotics as prophylactic protection and growth promoter across industries, such as animal husbandry and aquaculture, has also accelerated the emergence of resistance in many parts of the world (Wassenaar, 2005; Defoirdt et al., 2011; van Boeckel et al., 2019).

Abbreviations: ABR, Antibiotic resistance; AMR, Antimicrobial resistance; L1, First language; SSI, Socio-scientific issues; SSR, Socio-scientific reasoning; RBPD, Role-based panel discussion; WHO, World Health Organization.

Without effective action to reverse current trends, the rise of antibiotic (multi-)resistance can lead to antibacterial agents that are less effective and potentially useless. Resistance to one specific antibiotic agent can lead to resistance to a whole class of antibiotics using a particular functional mechanism (Magiorakos et al., 2012). In addition, the development of new types of antibacterial medicines for clinical use, especially medicines that are effective against multi-resistant strains of bacteria, remains meager (Levy and Marshall, 2004; Brown and Wright, 2016).

These circumstances have triggered the development of coordinated and comprehensive national, European, and global action plans to face this problem. A common goal of these efforts is to improve awareness and understanding of the responsibility for individual and collective actions through effective communication, education, and training to create and promote circumstances for behavioral changes (World Health Organization, 2015). Indeed, guidance for antibiotic usage should be developed, according to Sharland et al. (2018), to meet the United Nations' Sustainable Development Goals, particularly the aims for good health and well-being (target three). Future generations of scientifically literate antibiotic users need to understand the role that particular stakeholders play in producing, prescribing, and using antibiotics to decrease ABR (World Health Organization, 2015; O'Neill, 2016). Cultural and conceptual knowledge, as well as numerous capacities and skills about ABR, are vital for improving health literacy (Sørensen et al., 2012; Hoffmann et al., 2014) and microbiology literacy (Timmis et al., 2019) in broader society. In conclusion, optimizing antibiotic use to reduce the impact and limiting the spread of resistance, especially multi-resistance, remains manifold and complex.

The rise of antibiotic-resistant bacterial infections affects all members of a community or society and is driven by many interconnected factors. As with other issues of human concern that the media is frequently showcasing, ABR is a significant, open-ended, and multifaceted contemporary societal issue that incorporates many disciplines and knowledge domains. Hence, antibiotic resistance as a global phenomenon offers an excellent entry point for dealing with socio-scientific issues (SSI) instruction in class to contribute to the scientific literacy development of students (Roberts and Bybee, 2014).

Socio-scientific issues classroom instruction represents a science teaching approach that anchors a comprehensive real-world societal issue with conceptual, procedural, or technical links to science as a context for learning (Sadler, 2004). By definition, SSI as curriculum practice entails: (i) participation in dialog, discussion, debate, and argumentation about personally relevant problems through evidence-based reasoning; (ii) the use of evidence from sciences as well as other disciplines to inform decisions; (iii) some degree of moral reasoning and ethical evaluation; and (iv) the development of virtue and character aimed in the long term (Zeidler, 2014).

Research has revealed that teaching about real-world contexts by serving students' interests and employing personally relevant issues could increase engagement among learners (Sadler, 2009). A substantial body of literature has documented that SSI

approaches ought to successfully support students in acquiring desired educational objectives, including interest and motivation in learning science (Albe, 2008; Romine and Sadler, 2016); skills in science practices (Sadler et al., 2007), such as reasoning (Sadler and Zeidler, 2005) and argumentation (Evagorou and Osborne, 2013); and epistemic understandings of science (Eastwood et al., 2012; Khishfe et al., 2017). Despite this progress, "there have been fewer advances in understanding how SSI can be productively incorporated in learning environments," as Sadler et al. (2017) noted.

Building on the methodology of "educational design research" (McKenney and Reeves, 2019), we intended to frame learning conditions focusing on engaging students to negotiate the scientific and social connections inherent in a hot spot of public health. To design the implementation of SSI-based instructional activities, we drew from both an SSI framework described by Presley et al. (2013) and a model for SSI teaching and learning proposed by Sadler et al. (2017; see Classroom Procedure section; **Figure 1**). The SSI teaching and learning instructional model posits three phases (Sadler et al., 2017):

a. The first phase involves students exploring the focal issue;
b. The second phase corresponds with the main body of teaching and learning experiences, including student engagement with science ideas and higher-order practices, such as argumentation, decision-making, and socio-scientific reasoning (SSR). Following the theoretical construct conceptualized by Sadler et al. (2007), SSR implies typical kinds of reasoning contained in most SSI. Accordingly, SSR consists of four epistemological traits: (i) recognizing the inherent *complexity* of SSI; (ii) examining issues from *varied perspectives*; (iii) appreciating that issues are subject to ongoing *inquiry*; and (iv) possessing *skepticism* in the examination of potentially biased information (Sadler et al., 2007);
c. The third and final phase covers a culminating exercise where the students synthesize their learning experiences with the issue under investigation.

As ABR is multidimensional, the issue demands consideration from different perspectives and dimensions. For this purpose, we leveraged role-playing (Howes and Cruz, 2009; see **Supplementary Table S2**) within our SSI-embedded curriculum unit as a productive context for engaging students in learning opportunities that connect school experiences with a real societal debate. Ødegaard (2003) argues that "the role-play presents a learning opportunity which focuses both on scientific epistemology and scientific personality." Like an imitation of a societal practice, role-playing exercises have been widely recommended. Scholars and practitioners have revealed numerous advantages of learning through role-play classroom approaches. These advantages include enabling students to potentially gain and improve an understanding of multiple perspectives on issues at both macro and micro levels, enhancing emotional engagement with matters of human concern, and developing

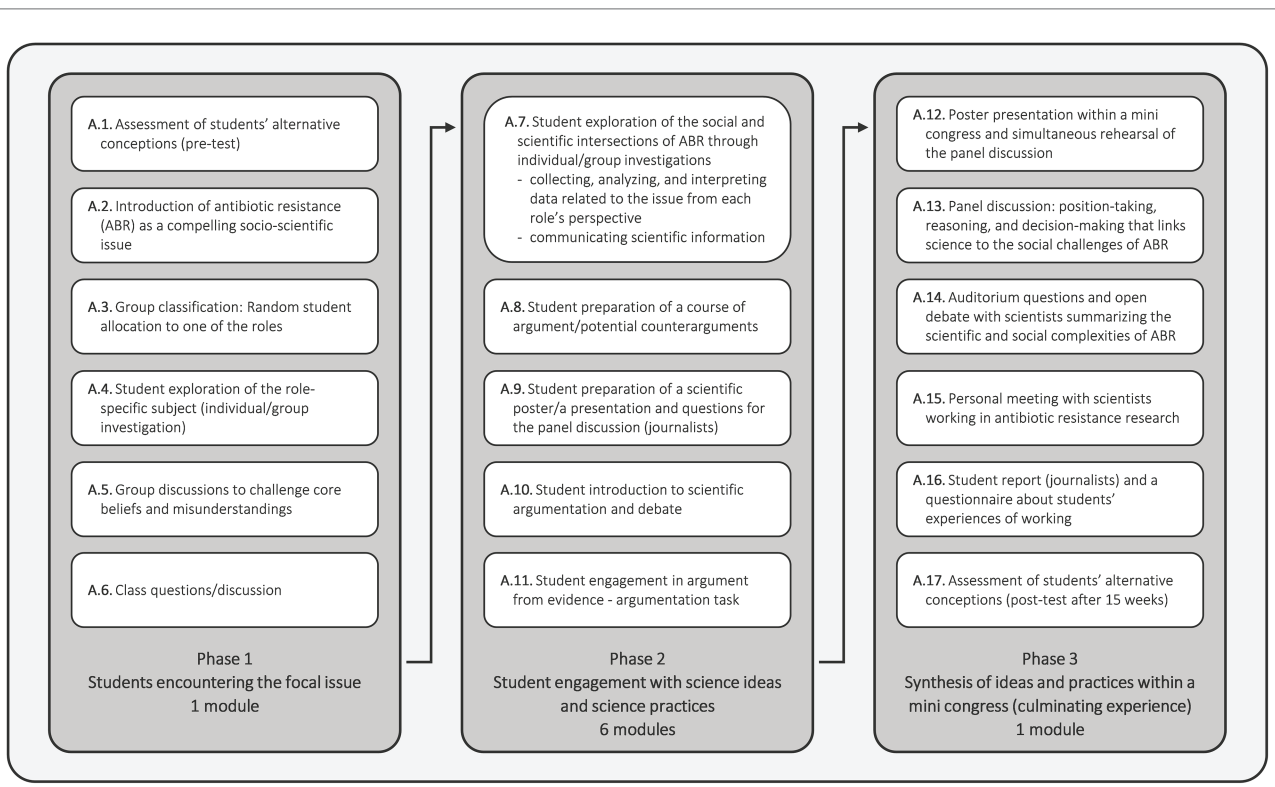

FIGURE 1 | Overview of the classroom procedure of the SSI-embedded role-playing curriculum unit consisting of eight modules. A, activity.

and bettering individual and interpersonal skills (Bolton and Heathcote, 1999; Van Ments, 1999; McSharry and Jones, 2000; Simonneaux, 2001; Howes and Cruz, 2009; Belova et al., 2015). The active involvement of students in reinterpreting information and data from a different perspective facilitates a more stable anchoring of the knowledge gained (Duveen and Solomon, 1994).

There is evidence that this learner-centered approach is useful in implementing real-world contexts in science education (Simonneaux, 2001; Agell et al., 2015; Belova et al., 2015), for example, by utilizing role-based panel discussions (Vrabl and Vrabl, 2012). In a role-based panel discussion (RBPD), a variant of a role-play, the students act in place of the assigned position taken in front of an audience as panelists (see Phase 3: Synthesis of Ideas and Practices Within a Mini Congress, Providing a Culminating Experience section; **Figure 2B**). As student-active tools, role-playing in general and RBPD more particularly raise no claim to a solely clear-cut conclusion of the problem or debate. The emphasis here is not the solution of a problem, but the recognition and understanding of the underlying structural conflict patterns from multiple perspectives (Ødegaard, 2003), such as socio-political dynamics of controversially discussed microbiological-related issues (Vrabl and Vrabl, 2012). However, the application of societally oriented role-playing activities in science classrooms seems to remain limited (McSharry and Jones, 2000; Hofstein et al., 2011).

Educational studies that contextualize ABR lessons related to SSI instruction have often only focused on a single or a few aspects. Teaching and learning about evolution, natural selection, or modeling has been often emphasized (Friedrichsen et al., 2016; Williams et al., 2018; Peel et al., 2019). The present study aims to contribute to this research area by investigating students' engagement in examining and negotiating scientific and social ramifications inherent in this complex issue. These social dimensions significantly shape the issue and interrelate habitually with how the underlying science is applied or interpreted (Saunders and Rennie, 2013). In this work, we report on students' accounts of SSI teaching and learning in a role-playing classroom setting, illuminating ABR as a multidimensional and multi-perspective field of health, social, economic, and ecological relationships and discourses. Embedded in SSI-based instruction, the research question guiding this investigation was: How do students experience a role-playing learning environment which addresses the complex interplay of societal and scientific issues related to the phenomenon of antibiotic resistance?

MATERIALS AND METHODS

Ethics Statement

This study was carried out following the recommendations of the Internal Review Board for Ethical Issues of the University of Innsbruck (Austria). Permission for participation and ethical approval of all procedures was obtained and approved by the provincial government's school authorities, namely the "Bildungsdirektion für Tirol" as well as the "Bildungsdirektion für Vorarlberg," which are the institutions that approve studies involving school students in the Austrian provinces Tyrol and Vorarlberg. The corresponding ethical approval code is 113.08/0067-allg/2018. A letter of information was provided to parents, guardians, teachers, and the headmasters prior to the classroom activity and the surveys. Signed guardian consent forms were obtained, allowing the students to voluntarily participate in the study with the possibility of withdrawal at any time; no refusals were registered. Parents were prompted to advise the lead researcher if they did not want to disclose sensitive information of their minor child under the age of 18, which happened in three cases (two cases in study group A and one case in study group B). These students did not provide any information concerning age and first language (L1; for the profile of the study groups, see **Table 1**). The surveys were conducted anonymously to protect student data and privacy. In this article, written informed consent to publish any potentially identifiable images or data was obtained from the students' guardians and full-age students (aged 18 years or older), respectively. In order to avoid influencing the students' answers, none of the researchers and project team members were familiar with the participants.

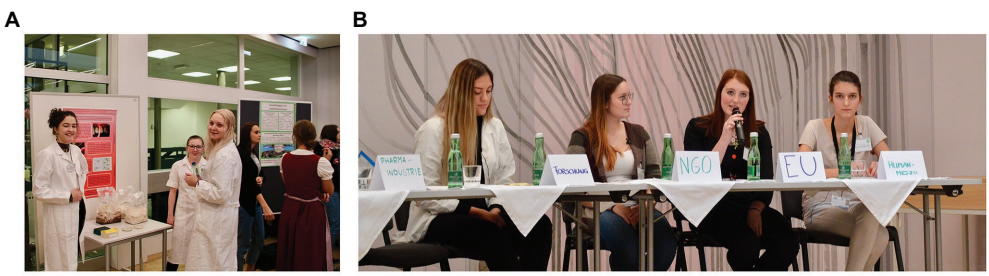

FIGURE 2 | Impressions of the poster presentations at the mini congress **(A)** and the panel discussion **(B)**. **(A)** Three students in lab coats are waiting for visitors to present their poster about novel candidates for antibiotics and displaying on the desk bags and Petri dishes with different fungal cultures as a source of antibiotics. **(B)** Students vividly discussing the crucial factors of antibiotic (multi-)resistance in their respective roles (from left to right): a publicly-funded scientist, an activist of a non-governmental organization, a member of the European Commission, and a physician. Students representing the pharmaceutical industry, the agriculture, the World Health Organization, or the hosting journalist(s) are not depicted in the photograph. For the students' experiences in the respective roles, see Results section; **Table 3**.

TABLE 1 | Number, sex, and age of participants.

Group	Grade level	Number of students	Females		Males		No entry	M_{age}	SD_{age}	Age range	No entry
		n	*n*	%	*n*	%					
A	11	26	25	96	1	4	-	17.42	0.58	17–19	2
B[1]	9	26	9	35	17	65	-	14.88	0.78	14–18	1
C[1]	10	19	8	42	11	58	-	15.26	0.45	15–16	-
Total	9–11	71	42	59	29	41	-	15.88	1.31	14–19	3

[1]*This group participated in the science communication project but was not analyzed in this paper.*

Preliminary Pilot Activities

At a higher education level, we built on experiences with role-playing and the staging of role-based panel discussions for demonstrating socio-political dynamics of controversial topics within microbiology, such as (xeno)estrogens in wastewater (Vrabl and Vrabl, 2012). With the assistance of biology student teachers who were about to graduate, we employed this approach in two pilot projects (held in 2016 and 2017) that engaged senior high school students in the discussion of the controversial topic of ABR.

The pilot activities were performed in two grade 11 biology and environmental education classes from two Upper Secondary Schools (senior high schools) in Tyrol (Austria). Based on the insights and data gained from the pilot classes, which were not used for the analysis of this study, the role-based classroom setting was slightly refined and expanded by a mini-congress with a poster exhibition (see Phase 3: Synthesis of Ideas and Practices Within a Mini Congress, Providing a Culminating Experience section; **Figure 1**, A.12.; **Figure 2**). It was deemed essential to rebalance the roles that the students undertook. For example, *university research* was integrated as an independent role because, in the previous distribution of the responsibilities, it was felt that the pharmaceutical industry appeared as the sole innovation driver for new antibiotic substances, which gave the students representing the pharmaceutical manufacturers a certain unassailability. However, universities have made and continue to make decisive contributions to research into novel antibiotic substances, alternative therapies, or resistance mechanisms. Coates et al. (2011) emphasized the role of university research in stemming the tide of ABR and argued that "universities should be encouraged to rebuild their antibiotic discovery sectors and to replace lost skills in this field. Clearly this will take decades, but antibiotic discovery is something that will need to be continued into the foreseeable future." The unique characteristics distinguishing university research from its competitors, public funding issues, or its underlying structural problems and challenges, all these aspects are subsumed with the redefined role of the *publicly-funded scientist*.

Most of the pre-service teachers and the biology teacher of study group A have been involved in at least one of these pilot interventions. Thus, the project members, i.e., the students' mentors, were thoroughly acquainted with the procedure and the role-specific subject matters. In the current study, particular emphasis was given to embed the context of SSI teaching and learning into the role-playing classroom environment.

Classroom Procedure

We describe the curriculum unit using Sadler et al. (2017) model of SSI teaching and learning as a framework. **Figure 1** provides an overview of the schedule of the curriculum unit. **Supplementary Table S1** presents a lesson plan for the curriculum unit. We designed the role-playing classroom environment to promote the students' contextual understanding of the scientific and social concepts and processes underlying antibiotic resistance. Each SSI module required two to three consecutive lessons (50 min each) at least once a week, i.e., a total of 16 h of in-class instruction (see **Figure 1**; **Supplementary Table S1**). As an additional motivation, students were advised that enrollment in this classroom setting would contribute to their biology grade. The assessments of the students were carried out by their biology teacher.

Groups of three to four students were assigned to examine one ABR role perspective, and each group was accompanied by a mentor, who had thoroughly studied the perspective of a specific role to employ critical analysis and skepticism. Through online student mentoring and personal group meetings, each student group was explicitly able to draw on the support and advice of a supervisor who guided the workflows throughout the curriculum unit. Thereby, the emphasis was placed on (i) advising and supervising the workflow; (ii) instruction for subject-specific questions; and (iii) providing feedback on the quality of the student assignments. The guidance offered to each group aimed to ensure that the students would not be distracted or overwhelmed when working with a complex issue and with an outcome that cannot be predefined.

The class' biology teacher, a group of scientists, and biology student teachers from the University of Innsbruck offered the participants content knowledge to scaffold student learning and higher-order practices. Supplementary information covered fields of microbiology, immunology, molecular biology, and genetics, tempered with knowledge about the social processes necessary to understand ABR. Subject-specific contents were reinforced and reiterated on multiple occasions, and this was done outside the classroom as well.

This mentoring ensured that the students were generally familiarized with the basic underlying concepts across several levels of biological organization, i.e., cellular, organism, and population levels. More specifically, they were encouraged to develop deeper scientific understandings of the mechanisms of ABR aligning with the social problems and consequences antibiotic-resistance bacteria may cause. Student activities also

included a field trip to the Department of Microbiology of the University of Innsbruck before the closing SSI phase for those allocated to the publicly-funded scientists' group (see **Supplementary Table S2**).

Phase 1: Students Encountering the Focal Issue

The curriculum unit began with presenting a compelling issue as a means of contextualizing the ensuing classroom activities requiring student engagement and commitment to active discovery. As teaching about bacteria, antibiotics, and resistance provides common misunderstandings (Simonneaux, 2000; Gregory, 2009; Byrne, 2011; Brookes-Howell et al., 2012; Bohlin et al., 2018), the alternative conceptions of the students were considered and elicited in this introductory sequence (see **Figure 1**, A.1.).

During this initial experience with the personally relevant issue, i.e., one module (see **Figure 1**, A.1.–A.6.), the students were confronted with demonstrations of newspaper headlines, articles, and visual presentations of the current scientific debate to capture their attention (see **Figure 1**, A.2.). Through formal instruction, the students then were familiarized with and reminded of basic facts and contexts related to bacteria, antibiotics, and resistance. This teaching sequence provided the students with the theoretical background, including a fundamental vocabulary, to enable better comprehension of information obtained through personal investigation and small group negotiation activities.

Eight roles were designed to expose the range of societal intersections involved in the highly complex issue of antibiotic-resistant bacteria (see **Supplementary Table S2**). Wherever possible, we sought to link the roles to the socio-cultural context of Tyrol/Austria to make the scope of ABR relevant and engaging for the students:

- The publicly-funded scientist (i.e., microbiologists in particular);
- The representative of the pharmaceutical industry (i.e., executives of a drug manufacturing company);
- The livestock farmer;
- The physician (i.e., clinicians and general practitioners);
- The activist of a non-governmental organization (i.e., individuals concerned about the national or international scope of antibiotic policies);
- The representative of a supranational organization (i.e., a panel of the European Commission);
- The international public health official (i.e., a representative of the World Health Organization);
- The journalist.

The students were randomly allocated to one of the roles mentioned above (see **Figure 1**, A.3.). Subsequently, they conducted first individual and group investigations to explore the role-specific subject matter (see **Figure 1**, A.4.). As scaffolds, the students received pre-structured information in the form of a role-specific assignment sheet (e.g., for the pharmaceutical industry representatives, see **Supplementary Figure S1**), assisting them in preparing for their respective roles by providing an evidence base and lines of reasoning. A small collection of selected documents and online sources provided the learners with further information. **Supplementary Table S2** illustrates each role's chain of potential argumentation and selected sources.

Socially shared class activities (see **Figure 1**, A.5.) were used to challenge the students' core beliefs and misunderstandings. Class questions and discussion (see **Figure 1**, A.6.) provided the learners with a place where they can expand on their existing knowledge about the issue, concluding phase one.

Phase 2: Student Engagement With Science Ideas, Science Practices, and Socio-Scientific Reasoning Practices

The second phase of the curriculum unit consisted of six modules (see **Figure 1**, A.7.–A.11.). These modules were intended to promote inquiry to encourage students with science practices that reflect the complex social and scientific intersections. We pursued to encourage students to employ the following practices: (i) collecting, analyzing, and interpreting data; (ii) communicating scientific information by using information and communications technologies; and (iii) engaging in argument from evidence. To allow students to develop better understandings in all areas, we offered the learners occasions to exchange specialist content knowledge and report their findings among their classmates. During the assignments (see **Figure 1**, A.7.–A.9.), the students were in contact with their mentors, both in person and online.

Each student group was expected to organize and analyze evidence and sources throughout several weeks (see **Figure 1**, A.7.; **Supplementary Figure S1**), including evaluating the validity and reliability of evidence, to support the stakeholder position to which their team was assigned. More specifically, the students aimed to properly understand the role they had assumed through investigating, both individually and in groups, media and Internet resources pertaining to the various stakeholders. These sources covered scientific articles, scientific and governmental reports, and presentations of original experimental and epidemiological data prepared for broader audiences.

This exercise (see **Figure 1**, A.7.) resulted in the formulation of a full course of argument and a chain of potential counterarguments informed by ideas and commitments from each role's perspectives (see **Figure 1**, A.8.). Additionally, each learner team created a scientific poster collaboratively illustrating their stakeholder position except for the student group representing the science journalists (see **Figure 1**, A.9.; **Figure 2A**). Both assignments (see **Figure 1**, A.8., A.9.) were aimed to encourage students to develop and deepen their understanding and informed opinions based upon reliable evidence backing each role's standpoint.

The journalists' group took a unique position within these two activities (see **Figure 1**, A.8., A.9.). These students collected the chain of argumentation from each representative group to familiarize themselves with the stakeholders' standpoints. On this basis, the journalists prepared a presentation for the panel discussion's opening that highlighted the features of the emergence and dissemination of ABR and the complex interplay of its accelerators. Besides, this learner group developed a list of

questions to moderate the RBPD. These questions were intended to point out the contradicting opinions and divergent interests of the panelists.

In phase 2, discussion activities were used to engage students in dialog and support their argumentation and reasoning skills in a module lasting three lessons (see **Figure 1**, A.10., A.11.). First, the students were introduced to scientific argumentation (see **Figure 1**, A.10.). The students then had to deal with an oral and written argumentation task, which aimed at encouraging them to apply their reasoning skills and utilize their newly acquired knowledge (see **Figure 1**, A.11.) reflecting the social and scientific dimensions of antibiotic resistance adapted from Rafolt et al. (2019b).

In the meantime, further school classes were introduced in the subject, explaining the problems related to the spread of antibiotic-resistant bacterial diseases. As congress participants and attendants of the panel discussion (**Figure 1**, A.12.–A.14.; **Figure 2**), these students (n = 139 in total; study group A: n = 48; study group B: n = 48; study group C: n = 43) constituted the audience for the culminating exercises (see Phase 3: Synthesis of Ideas and Practices Within a Mini Congress, Providing a Culminating Experience section). First, in small groups of three to four, the students were allocated to one of the stakeholder groups (see **Supplementary Table S2**). As part of a series of structured activities, the students explored data and information related to bacterial diseases and ABR. Next, they prepared questions for the plenary discussion carried out after the penal discussion (**Figure 1**, A.14.) from the perspective of the relevant stakeholder group. After studying the views of a specific stakeholder group, the students reported their findings and insights to their fellow students.

Phase 3: Synthesis of Ideas and Practices Within a Mini Congress, Providing a Culminating Experience

In the final SSI phase (see **Figure 1**, A.12.–A.17.), students synthesized their learning experiences related to the claims made and corresponding to the questions posed within a mini congress, including a poster presentation (see **Figure 1**, A.12.; **Figure 2A**) and a panel discussion (see **Figure 1**, A.13., **Figure 2B**). This phase challenged the students to create and justify recommendations for limiting the emergence and spread of antibiotic-resistance with human health risks. The culminating activities were intended to reveal the social dimensions of ABR. The students were able to: (i) demonstrate their awareness of how scientific ideas and practices encountered affect stakeholder group perspectives and (ii) foster decision-making that links science to social challenges. Likewise, students applied their scientific understandings and role-specific knowledge to grapple with some of the societal challenges and problems that emerge from ABR. **Figure 2** presents images from the mini congress, including a poster presentation and the closing panel debate experience.

While two to three randomly selected members of each student group presented their scientific posters by communicating scientific information to peers and teachers (see **Figure 1**, A.12.; **Figure 2A**), another randomly selected member of each student group was nominated to represent and defend the assigned position in the panel discussion (see **Figure 1**, A.13.; **Figure 2B**). These students rehearsed the organizational procedure of the panel discussion before they went into the discussion.

Following the poster presentation, the panel discussion took place (see **Figure 1**, A.13.; **Figure 2B**). Requisites (e.g., white coats for the physicians; traditional working garments of Austrian farmers; suits and ties for the politicians; and laboratory coats for the scientists) were provided to facilitate role assignment (see **Figure 2**). One or two students belonging to the journalists' group moderated the panel discussion, which started with an introductory presentation about the focal topic (see **Figure 1**, A.9.). The hosts, i.e., moderators, were then responsible for leading the debate by questioning the panel members, giving the floor to someone, or calling them to order when necessary.

In the panel discussion (see **Figure 1**, A.13.; **Figure 2B**), the students outlined antibiotic resistance at an individual, national, or international level from each role's perspective. More specifically, the discussants negotiated their positions, sometimes illustrated by studies to substantiate their specific area, and debated the responsibility for the current situation of antibiotic-resistant bacteria, explored alternatives, and even sought novel or creative solutions. The discussion ended with a closing statement by each panelist in which they emphasized why their approach to contain the worldwide emergence and spread of ABR may be sensible.

After that, the audience forming students questioned the panel members within an open debate (see **Figure 1**, A.14.). As ABR constitutes a complex problem that lacks simple, clear-cut solutions, real-life scientists from the field of microbiology summarized the panel discussion and the subsequent plenary questions (see **Figure 1**, A.14.). These experts highlighted that knowledge of the emergence and spread of antibiotic-resistant bacteria needs to be highly contextualized within a complex social, political, and economic context. After both discussions, all students were given a chance to talk to microbiologists and pharmacologists working in novel antimicrobial substance research (see **Figure 1**, A.15.). The reason behind this was to provide meaningful, authentic every-day life experiences concerning antimicrobial research.

The students representing the journalists wrote an article summarizing the students' investigations on the focal issue for a popular science magazine (see **Figure 1**, A.16.). After 15 weeks, the students repeated the survey to elicit their conceptions on ABR (see **Figure 1**, A.17.).

Participants

So far, secondary school (senior high school) students ranging from grades 9 to 11 from three publicly-financed schools located in urban areas in Tyrol (study groups A and B) and Vorarlberg (study group C) participated in the curriculum unit as part of a school-based science communication project. For this work, we analyzed the classroom experiences of the grade 11 students in detail (i.e., group A). **Table 1** summarizes the profile of participating study groups. We selected the participant

schools due to their particular focus on teaching natural and human sciences. We assumed that the students (age range: 14–19), who have chosen this specialization, have learned comparable subject-specific content at school and are motivated learners. A representative comparison of the samples was not sought. All students participated in the intervention within their regular biology classes (at least 100 min. per week).

In the autumn 2018 semester, the first investigation (study group A) consisted of 26 grade 11 students (aged 17–19, 96% females) from a Secondary School for Economic Professions (College for Higher Vocational Education). Twenty-five female students and one male student with a mean age of 17.42 ± 0.58 years took part in the curriculum unit throughout 8 weeks consecutively. Due to the tradition of the school type in general and the school's history more specifically, the vast majority of students attending the participating school were girls. German was the first language for all students, but one student spoke Turkish fluently (see **Table 1**). The school has an influential culture that emphasizes cross-disciplinary teaching with cross-curricular connections and student-orientated lesson design, encouraging students to work and learn in a self-reliant manner. We chose this study group for the implementation of our SSI curriculum unit because: (i) The biology teacher draws on three decades of teaching experience and (ii) took part in previous interventions; (iii) the school directorate immediately embraced the request to collaborate. Furthermore, students from this instructional level (grade 11) were recruited because this is the academic year when ABR-related issues are particularly accentuated (Federal Law Gazette II No. 340, 2015). As the students were planning to take their final exams the following year, we deliberately sought to motivate them to consolidate and enlarge content knowledge and to employ their written and verbal argumentation skills.

Data Collection

Qualitative data were collected by using a paper-and-pencil task requiring written open-response explanations to assess the situational characteristics of students' experiences of working with a SSI-based curriculum unit using role-playing to negotiate ABR. **Table 2** provides the questionnaire-based assessment instrument with six items. For 2 weeks upon completion of the classroom activities (winter holiday period), the grade 11 students ($n = 26$) were asked to explicitly describe their personal experiences of working with this teaching and learning environment. All students were instructed to answer as completely as possible. Those students who were absent when the collection of student responses took place handed in their open questionnaires later. The biology teacher forwarded the inquiries to the first author to ensure the anonymity of the study participants.

Data Analysis

A qualitative case study design was used to answer the given research question (Stake, 2010). Participants' contributions were iteratively examined for common features applying an inductive category development (Mayring, 2015). The statements of the students were prepared and examined in accordance with the following four steps:

1. Preparation of raw data: transcription of students' written essays;
2. Editing the transcripts, i.e., transfer of students' statements into a grammatically correct form;
3. Arranging and coding students' testimonies, i.e., a summary of identical or similar statements to thematic groups;
4. Explication, i.e., interpretation of the statements and identification of learning outcomes;

Using the computer-assisted qualitative data analysis software MAXQDA™ (release 20.0.6), the data collected were codified and organized into themes (Rädiker and Kuckartz, 2019). Finally, two other qualitative researchers independently reviewed parts of the transcripts for eliciting the learning experiences of the students. Although it is acknowledged that some students demonstrated particularities in their thinking, we have sought to produce a generalization of the individual student statements. In our attempt to understand the students' experiences with a learning environment, we found it crucial to go beyond subjective experiences and, therefore, strived to address the collective understanding of student responses. The interpretations are based on the German transcripts; quotations have been subsequently translated. All names have been pseudonymized.

TABLE 2 | Questionnaire-based assessment tool to examine students' experiences of working with the SSI-embedded curriculum unit using role-playing to address antibiotic resistance.

No.	Item[1]
(1)	Describe the *learning experiences* made while being engaged in the SSI-embedded role-based classroom setting addressing antibiotic resistance.
(2)	Describe the *learning progress* made while being engaged in the SSI-embedded role-based classroom setting addressing antibiotic resistance.
(3)	Describe the *learning outcomes* made by participating in the SSI-embedded role-based classroom setting.
(4)	Explain *whether and why* this SSI-embedded role-based setting may or may not be *recommended* to fellow students.
(5)	Give reasons if some *activities* of the SSI embedded role-based curriculum unit might be *changed* if the setting is repeated.
(6)	Describe whether or not the *outcome of the group work* was satisfactory.

[1]*The questions were administered in German.*

RESULTS

Inductive text analysis on the written contributions of the grade 11 students revealed cognitive and affective outcomes. **Table 3** indicates the frequency of categories of learners' experiences of working with the SSI-based curriculum unit. According to the students' self-reported experiences, the majority found the content exciting and related to a current societal challenge. Most students claimed to have learned "a lot," according to their judgment. As the students stated here, the curriculum

TABLE 3 | Selected categories of students' experiences in the course of the SSI-based curriculum unit.

Category[1]	Frequency (*n* / 26)
The student recommended the curriculum unit to classroom fellows.	24 / 26
The student indicated to have learned "a lot".	21 / 26
Exciting and personally relevant content.	20 / 26
Role-playing facilitated the student's contextual understanding.	19 / 26
The student was satisfied with the group work.	19 / 26
The assignments were labor-intensive.	18 / 26
Peripheral influences affected student engagement.	12 / 26
Student engagement in science practices.	12 / 26
Certain activities required a high degree of student self-organization.	11 / 26
The student referenced individual decision-making regarding antibiotic consumption.	8 / 26
The student reported inconsistencies regarding the division of labor within the student group.	8 / 26
Communicating data and information in front of an audience was a demanding task.	7 / 26
The student struggled in assuming the role.	7 / 26

[1]*The categories are sorted by the number of students mentioning this experience.*

unit is useful for learning new facts and generic skills. The unit "offers a variety to the tiring every-day school life," as Nora emphasized. Elisa also elucidated: "It was interesting to be in such a close contact with scientists and microbiologists and to get to know their job better."

The students noted that the curriculum unit revealed individual and societal as well as national and supranational levels of conflict, such as individual and collective health care decision-making. Addressing the causes and possible solutions to the emergence and spread of antibiotic-resistant bacteria, the students perceived that working with this SSI is relevant for their every-day lives and future. Some students considered to have benefitted from the knowledge gained. Regarding the enhancement of individual health outcomes, students stated that they had become more aware when taking antibiotics. A few students noted that they would refrain from consuming mass-produced meat because it might be contaminated with antibiotic-resistant pathogens.

In large part, students found the experience of working with this classroom approach, which leverages role-playing within SSI teaching and learning, and its assignments labor-intensive. Most of the students underestimated the time required to solve the tasks (see Phase 2: Student Engagement With Science Ideas, Science Practices, and Socio-Scientific Reasoning Practices section; **Figure 1**, A.7.–A.9.; **Supplementary Figure S1**). Despite a weeklong working period, some students reported that this interval was insufficient to engage with the subject from various perspectives accurately, deeply, and satisfyingly. That is why students wished for more SSI modules because some struggled to complete all tasks during the lessons offered. Elsa commented: "Such a classroom approach requires much time. The students have to deal with the topic in detail. In any case, sufficient time must be made available in the school lessons to fulfill the required tasks." Some participating students indicated that peripheral influences affected their engagement in the curriculum unit. They reported that the classroom unit's implementation coincided with assignments and tests that had to be carried out for other subjects. This circumstance consequently reduced, for some students, the motivation for participating. Therefore, the students demanded to have a say in the selection of the classroom approach's time of implementation. The willingness of the students to complete assignments out of school was low.

The starting phase (see Phase 1: Students Encountering the Focal Issue section; **Figure 1**, A.1.–A.6.) was essential for sparking interest and stimulating the search for and evaluation of role-specific data and information. The statements of the participants showed that students found it initially challenging to empathize with the role they took on (see **Supplementary Table S2**). For example, the students representing the publicly-funded scientists initially struggled in assuming their role because they knew very little about a microbiologist's profession, as one student elucidated. The visit to the microbiological laboratories at the University of Innsbruck and the interaction with scientists in person facilitated the students in understanding their role. This experience was also relevant for students who had undertaken other roles: the longer they dealt with the topic from their roles' perspectives, the easier it became to represent the role.

The examination of the subject matters through individual and group investigations (see **Figure 1**, A.4., A.7.–A.9.), and the taking up of specific roles had a very personal effect. Some students declared that the role-playing supported a secure anchoring of the content knowledge. Others resounded the deep rooting of the knowledge acquired as they expressed that the culminating experiences (see Phase 3: Synthesis of Ideas and Practices Within a Mini Congress, Providing a Culminating Experience section; **Figure 2**), i.e., the poster presentation (see **Figure 1**, A.12.; **Figure 2A**) and the panel discussion (see **Figure 1**, A.13., A.14.; **Figure 2B**) in particular, also contributed to the subject matters becoming a longer-term memory. Luca recapitulated: "Role-playing remains in memory." Students mentioned that they discussed the issue with their families, friends, and acquaintances outside the classroom. These discussions supported the assumption of the role and the elaboration of the assignments.

Observing ABR from different perspectives and dimensions helped the students to develop a proper contextual understanding to face antibiotic resistance, as students emphasized. Role-playing thus facilitated the student's contextual understanding. Many students claimed that they inquired about several stakeholder positions, highlighting the focal issue's scientific and social intersections that make the problem challenging. Talking about examining ABR from various perspectives, Florine pointed out that "the role-playing activities provided me with lots of new information on the subject of ABR, both in general and in relation to the individual small groups. The inside views gained from the perspectives of the different roles were extremely informative and gave me a multifaceted idea of the rather questionable use of antibiotics in different areas." However, a few students argued that they gained role-specific knowledge

of ABR predominantly. Prominently, the students referred to the following circumstances on the promotion of ABR:

- The incorrect prescription of antibiotics through physicians;
- The unnecessary ingestion of antibacterial drugs by self-medicated patients;
- The high development costs and the related outsourcing of antibiotic manufacturing to lower its production costs;
- The restriction of antibiotic research conducted in academia due to funding cuts; and
- The unnecessary use of antibiotics in agriculture and husbandry to promote growth or prevent infections and their dissemination into the environment.

They also noted that they acquired an understanding of the social dynamics inherent in the reduction of ABR based on the different interests of various stakeholders. The increase in ABR with limited development of novel antibiotics requires a prudent, controlled, and appropriate use of these substances in all areas of application, as several students summarized.

Students' learning experiences indicated that the activities engaged them in SSR and higher-order practices, such as argumentation and authentic decision-making based on sound facts. Student work also provided experiences in teamwork, conflict management, and organization. For certain activities, such as student interaction with science ideas and practices (see Phase 2: Student Engagement With Science Ideas, Science Practices, and Socio-Scientific Reasoning Practices section; **Figure 1**, A.7.–A.9.), it required a high degree of student self-organization and autonomy. They were committed to taking responsibility for themselves and their group members. In some cases, the students reported that it was not easy for them to divide the work evenly among group members. Contacting the group supervisor when needed, provided the students with confidence in completing the assignments. Two students suggested that roles should not be assigned randomly (see Phase 1: Students Encountering the Focal Issue section; **Figure 1**, A.3.). Instead, students should have the opportunity to allocate the groups by themselves, as Valeria elucidated: "If the groups could be selected freely, there might have been a greater sense of community among classmates. The division of labor might have been fairer. Also, we would certainly be more motivated to work together after school." However, most students were satisfied with both their own and their group's achievement. According to several student testimonies, working in groups led to a more comprehensive understanding as opposed to examining SSI through individual investigations because "through group work, we have been able to do a more versatile elaboration of the topic than individually," as Franziska stated.

The students argued that they learned to organize, analyze, evaluate, and present relevant scientific data and information concerning the promotion of science practices (see Phase 2: Student Engagement With Science Ideas, Science Practices, and Socio-Scientific Reasoning Practices section; **Figure 1**, A.7.). "For our future professional careers, it was beneficial to practice evaluating the seriousness and credibility of various sources," as Nora mentioned. However, some students found organizing essential sources, identifying arguments, and judging the credibility and validity of scientific data challenging.

The intensive mentoring enabled the learners to contribute to the group and discussion activities, as several students stated. Others echoed this belief by emphasizing the importance of the group mentor's assistance, in particular, to establish a scientific poster (see **Figure 1**, A.9.). The students expressed that elaborating relevant arguments and counterarguments from each role's perspectives empowered them to argue for the assigned role's standpoint. The engagement with science and SSR practices helped prepare for the writing of a diploma thesis and the oral "Reifeprüfung" examination, i.e., their final school-leaving oral exams, in the following year.

Student statements showed that the presentation and communication of relevant information in front of an audience within the culminating experience was a demanding task (see **Figure 1**, A.12., A.13.; **Figure 2**). Concerning the panel discussion participants' selection, some students commented on being glad that they did not have to take this assignment and could present their posters instead. One student suggested waiving the random drawing of poster presentation and panel discussion participants. Instead, each student group should choose the roles for themselves in the culminating experience. Three students emphasized that they, for example, managed to get over the fear of communicating data and information to an audience, as they either had to exhibit their poster or participate in the panel discussion. A student panel member, Nadia, proudly described the experience of participating in the panel discussion as follows: "The concluding panel discussion was a new experience. In the beginning, I was very calm and did not worry at all. When we took our places and started discussing, I felt nervous and just wanted to leave. After the discussion started, it was still difficult at first, but it became easier with time. One came fully into the role. At some point, I did not realize that we were sitting in front of many people. Time also passed very quickly. It was a beautiful experience." According to two student panel discussion members' experiences, the rehearsal of the procedure also supported a self-confident appearance in the final debate.

Except for two participants, all students recommended this classroom unit to fellow students attending a life sciences specialization. However, the students could not estimate whether they learned more through this SSI-based classroom instruction than through conventional science teaching approaches.

DISCUSSION

According to the WHO (World Health Organization, 2018), "antibiotic resistance is one of the biggest threats to global health, food security, and development today." Although resistance occurs naturally, the misuse and overuse of antibiotics in human and animal health care is accelerating the problem. Antibiotics are often given without professional oversight, are taken by people with viral infections, are given as growth promoters in animals, or are used to prevent diseases in healthy animals (Ventola, 2015). Thus, one of the five strategic objectives in the "Global

Action Plan on Antimicrobial Resistance" is to improve awareness and understanding of this phenomenon to promote prudent antibiotic usage (World Health Organization, 2015).

In the last years, information and educational campaigns have been launched in several countries to increase antibiotic awareness (Cross et al., 2017). Waaseth et al. (2019) argued that "public knowledge is considered a prerequisite for appropriate use of antibiotics and limited spread of antibiotic resistance." In a recent study, Burstein et al. (2019) systematically identified public-directed interventions to promote antibiotic awareness in the United States. They found that multifaceted programs can change patient perspectives regarding antibiotic use. However, "most public messaging interventions focused on educating parents of young children through office-based posters and handouts," as they concluded (Burstein et al., 2019).

In the case-based study described by this paper, microbiologists and educational researchers came together to address the focal problem in the context of SSI teaching and learning (Presley et al., 2013; Zeidler, 2014; Sadler et al., 2017). In terms of the pedagogical practice, we designed an 8-week extended SSI-based classroom setting to engage students in examining and negotiating the scientific and social relationships of ABR (see Classroom Procedure section; **Figure 1**). We leveraged role-playing within the curriculum unit to illustrate controversial perspectives and dimensions of multiple stakeholders that habitually shape this deeply ramified issue with connections to science and society. To understand whether the curriculum unit successfully meets the goal of conveying ABR's multilayered interrelationships between social, political, economic, and scientific perspectives and dimensions, we drew on the methodology of "educational design research" (McKenney and Reeves, 2019). Retrieved from an open-response questionnaire (see Data Collection section; **Table 2**), grade 11 students' experiences of working with our SSI-based classroom unit using role-playing were qualitatively examined.

The present classroom intervention addressed juveniles and young adults in particular who could become parents, physicians, scientists, farmers, politicians, or could walk any other path of life. These individuals will likely be asked in the future to decide how and when to use antibiotics. By then, they may consider lessons learned and may make informed decisions. Many students argued that they learned "a lot" about antibiotics, the patterns for the emergence of antibiotic resistance, and different stakeholder perspectives on ABR. However, there is evidence that after 16 h of dealing with the focal issue, few students still have difficulties in explaining who is getting resistant (humans or bacteria) or how it happens that antibiotics become less effective in treating bacterial infections (Brookes-Howell et al., 2012). Despite a high level of knowledge of antibiotics and ABR among citizens, "there seems to be a knowledge gap when it comes to understanding the rationale behind the resistance problem," as Waaseth et al. (2019) similarly highlighted.

The evolution of ABR is multifactorial and deeply rooted in societal practice and individual ideas and beliefs. Thus, learning opportunities that shed light on the complexity of the phenomenon are needed in order to raise public awareness and proper conceptual understanding. Educational interventions designed by scientists, governmental organizations, or Centers for Disease Control often rely on traditional approaches. They predominantly address the scientific rationale that underlies the phenomenon while focusing on a particular goal, such as decreasing unnecessary antibiotic use or prescription (Burstein et al., 2019).

Hardly ever do educational interventions address links between science and society or allow learners to situate their actions in a broader societal context. Real-life intersections between science and society have been identified as SSI within the science education community already (Zeidler, 2014). Previous research has documented that SSI teaching and learning can have a positive impact on the science content learning of students (Klosterman and Sadler, 2010), on their understanding of the nature of science (Khishfe et al., 2017), and on their argumentation (Romine and Sadler, 2016), and the development of critical thinking skills (Sadler et al., 2007; Rafolt et al., 2019a). Overall, Hancock et al. (2019) emphasized that "SSI-based instruction has emerged as an effective way for students to contextualize their science learning within a complex social and political context." However, the implementation of SSI-based teaching in the every-day classroom remains limited because SSI instruction may be unfamiliar for many teachers (Ekborg et al., 2013). Besides, teachers frequently believe that their content knowledge is limited, and they experience a paucity of well-designed SSI-oriented curricular materials as well as limited support while trying to enact SSI teaching (Presley et al., 2013).

Socio-scientific issues curriculum units are challenging not only when it comes to designing a learning environment but also equally when the curriculum unit is put into practice. To overcome these barriers, microbiologists, pre-service biology teachers, and education researchers designed a SSI-embedded curriculum unit using role-playing (see Classroom Procedure section; **Figure 1**), including a plenary discussion within a mini congress setting (see Phase 3: Synthesis of Ideas and Practices Within a Mini Congress, Providing a Culminating Experience section; **Figure 1**, A.12.–A.14.; **Figure 2**). The role-playing classroom environment encouraged students to scrutinize the individual positions advocated and their dynamics with one another. It created identification and empathic understanding (Ødegaard, 2003). In providing meaning to content, role-playing allows the students to slip into a previously unknown position and deal intensively with their thoughts, attitudes, and interests, which may be of a private, public, political, or commercial nature concerning ABR. The designed roles (see **Supplementary Table S2**) represent diverse and multiple perspectives related to the emergence and spread of antibiotic-resistant bacteria, supporting students to learn more meaningfully about the relevant content. However, not everyone approves the task to put himself/herself into a role. Students of 17–19 years of age (see **Table 1**) have little or no experience with their assigned or other stakeholder professions, their daily tasks, or challenges. This unfamiliarity may make it difficult to either support or reject a specific routine in prudent antibiotic use. Hence, these students need increased support and detailed information on their role characteristics.

This observation led us to another prerequisite of successful SSI learning environments. A broadly based support system is helpful, not only for students but for teachers alike. Microbiologists, pre-service teachers, and education researchers supported participating teachers and students while being engaged in the classroom setting. The intensive mentoring enabled students to contribute substantially to the group and discussion activities. The group mentor's assistance was urgently needed to design a scientific poster and elaborate relevant arguments and counterarguments for each role's perspectives. Microbiologists introduced content knowledge, while education researchers offered a theoretically substantiated selection of teaching and learning tools. Teachers shared their practical expertise in scaffolding the student learning processes in-class. In this context, the question arises to what extent SSI learning approaches are feasible to be implemented on a broader scale or whether it should be a sustainable endeavor to support intensive collaboration between researchers and schools.

Students' self-reports about how they experienced working with SSI instruction are mainly positive (Ottander and Ekborg, 2012). However, Hancock et al. (2019) argued that the local and national contexts, school routines, and cultural traditions might have a considerable impact on student performance. All students reported that they had to take exams in several subjects while preparing for the poster presentation and plenary discussion. Accordingly, the time devoted to specific tasks (see Classroom Procedure section; **Figure 1**, A.7.–A.9.) was limited. Nevertheless, most students wished to have time to do more SSI modules and deepen their knowledge about ABR. The interactive learning environment helped students thrive and evoked their interest and motivation to learn more about ABR. However, the main obstacle that was difficult to overcome was linked to the time constraints of the students. Therefore, we highly recommend being aware of "Peripheral Influences" (Presley et al., 2013) that may also influence student learning in SSI-based units.

Overall, this study contributes to the enhancement of SSI teaching and learning in a real-world context with microbiology. In the study described for this paper, microbiologists and educational researchers presented a learning environment that has the potential to improve students' awareness and understanding of ABR. Microbiology offers a useful perspective and contributes to real-life contexts for SSI teaching and learning. Topics relevant to health and environmental education are tied together, and a significant challenge of the 21st century is negotiated (Fensham, 2012; Zeyer and Dillon, 2019). Concerning the case-based data reported, this work supports the assumption that our SSI-embedded role-playing classroom setting is well suited to support students in acquiring scientific knowledge about antibiotics and ABR and the complex interplay of social dimensions. However, Sjøberg and Schreiner (2012) have shown that there are gender differences in students' interests in learning about science topics, their experience with and views on school science, and their views and attitudes to science in society. In this study, mainly girls participated. The attitudes of males to this intervention might differ from what is reported. There are limitations caused by using an open question paper-and-pencil format. Students may interpret given questions differently and have varying competencies to express themselves in writing and drawing. Unfortunately, it was not possible to address long-term knowledge retention because participants were not available for interviews at any time later. However, this could be rewarding data to use in the subsequent use of this curriculum unit. Accordingly, additional research is required to assess whether the curriculum unit can help students develop higher-order thinking skills, such as argumentation, decision-making, or position-taking. Future research might also explore the extent to which students' SSR competencies improve while being engaged in the curriculum unit (Romine et al., 2020).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

PV conceptualized and designed the classroom unit together with CS. PV raised the funding. KS performed the study and gathered the data. KS wrote the first draft of the manuscript, which then was critically revised and rewritten by CS, SK, and PV. All authors contributed to the drafting of the figures, data analysis and interpretation, and read and approved the final version of the manuscript.

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REFERENCES

Agell, L., Soria, V., and Carrió, M. (2015). Using role play to debate animal testing. *J. Biol. Educ.* 49, 309–321. doi: 10.1080/00219266.2014.943788

Albe, V. (2008). When scientific knowledge, daily life experience, epistemological and social considerations intersect: students' argumentation in group discussions on a socio-scientific issue. *Res. Sci. Educ.* 38, 67–90. doi: 10.1007/s11165-007-9040-2

Alekshun, M. N., and Levy, S. B. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell* 128, 1037–1050. doi: 10.1016/j.cell.2007.03.004

Andersson, D. I., and Hughes, D. (2010). Antibiotic resistance and its cost: is it possible to reverse resistance? *Nat. Rev. Microbiol.* 8, 260–271. doi: 10.1038/nrmicro2319

Belova, N., Eilks, I., and Feierabend, T. (2015). The evaluation of role play in the context of teaching climate change. *Int. J. Sci. Math. Educ.* 13, 165–190. doi: 10.1007/s10763-013-9477-x

Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., and Piddock, L. J. V. (2015). Molecular mechanisms of antibiotic resistance. *Nat. Rev. Microbiol.* 13, 42–51. doi: 10.1038/nrmicro3380

Bohlin, G., Göransson, A., Höst, G. E., and Tibell, L. A. E. (2018). Insights from introducing natural selection to novices using animations of antibiotic resistance. *J. Biol. Educ.* 52, 314–330. doi: 10.1080/00219266.2017.1368687

Bolton, G., and Heathcote, D. (1999). "So you want to use role play?" in *A new approach in how to plan*. Stoke-on-Trent: Trentham Books.

Brookes-Howell, L., Elwyn, G., Hood, K., Wood, F., Cooper, L., Goossens, H., et al. (2012). "The body gets used to them": patients' interpretations of antibiotic resistance and the implications for containment strategies. *J. Gen. Intern. Med.* 27, 766–772. doi: 10.1007/s11606-011-1916-1

Brown, E. D., and Wright, G. D. (2016). Antibacterial drug discovery in the resistance era. *Nature* 529, 336–343. doi: 10.1038/nature17042

Burstein, V. R., Trajano, R. P., Kravitz, R. L., Bell, R. A., Vora, D., and May, L. S. (2019). Communication interventions to promote the public's awareness of antibiotics: a systematic review. *BMC Public Health* 19:899. doi: 10.1186/s12889-019-7258-3

Byrne, J. (2011). Models of micro-organisms: Children's knowledge and understanding of micro-organisms from 7 to 14 years old. *Int. J. Sci. Educ.* 33, 1927–1961. doi: 10.1080/09500693.2010.536999

Carlet, J., Collignon, P., Goldmann, D., Goossens, H., Gyssens, I. C., Harbarth, S., et al. (2011). Society's failure to protect a precious resource: antibiotics. *Lancet* 378, 369–371. doi: 10.1016/S0140-6736(11)60401-7

Carlet, J., Jarlier, V., Harbarth, S., Voss, A., Goossens, H., and Pittet, D. (2012). Ready for a world without antibiotics? The Pensières antibiotic resistance call to action. *Antimicrob. Resist. Infect. Control* 1:11. doi: 10.1186/2047-2994-1-11

Cassini, A., Högberg, L. D., Plachouras, D., Quattrocchi, A., Hoxha, A., Simonsen, G. S., et al. (2019). Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European economic area in 2015: a population-level modelling analysis. *Lancet Infect. Dis.* 19, 56–66. doi: 10.1016/S1473-3099(18)30605-4

Coates, A. R. M., Halls, G., and Hu, Y. (2011). Novel classes of antibiotics or more of the same? *Br. J. Pharmacol.* 163, 184–194. doi: 10.1111/j.1476-5381.2011.01250.x

Cross, E. L. A., Tolfree, R., and Kipping, R. (2017). Systematic review of public-targeted communication interventions to improve antibiotic use. *J. Antimicrob. Chemother.* 72, 975–987. doi: 10.1093/jac/dkw520

Davies, J., and Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* 74, 417–433. doi: 10.1128/MMBR.00016-10

Dawson, V. M., and Venville, G. (2010). Teaching strategies for developing students' argumentation skills about socioscientific issues in high school genetics. *Res. Sci. Educ.* 40, 133–148. doi: 10.1007/s11165-008-9104-y

Defoirdt, T., Sorgeloos, P., and Bossier, P. (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Curr. Opin. Microbiol.* 14, 251–258. doi: 10.1016/j.mib.2011.03.004

Depardieu, F., Podglajen, I., Leclercq, R., Collatz, E., and Courvalin, P. (2007). Modes and modulations of antibiotic resistance gene expression. *Clin. Microbiol. Rev.* 20, 79–114. doi: 10.1128/CMR.00015-06

Duveen, J., and Solomon, J. (1994). The great evolution trial: use of role-play in the classroom. *J. Res. Sci. Teach.* 31, 575–582. doi: 10.1002/tea.3660310510

Eastwood, J. L., Sadler, T. D., Zeidler, D. L., Lewis, A., Amiri, L., and Applebaum, S. (2012). Contextualizing nature of science instruction in socioscientific issues. *Int. J. Sci. Educ.* 34, 2289–2315. doi: 10.1080/09500693.2012.667582

Ekborg, M., Ottander, C., Silfver, E., and Simon, S. (2013). Teachers' experience of working with socio-scientific issues: a large scale and in depth study. *Res. Sci. Educ.* 43, 599–617. doi: 10.1007/s11165-011-9279-5

Evagorou, M., and Osborne, J. (2013). Exploring young students' collaborative argumentation within a socioscientific issue. *J. Res. Sci. Teach.* 50, 209–237. doi: 10.1002/tea.21076

Federal Law Gazette II No. 340 (2015). 340. Verordnung: Lehrpläne der humanberuflichen Schulen, Änderung der Verordnung über Lehrpläne für technische, gewerbliche und kunstgewerbliche Fachschulen, der Verordnung über Lehrpläne für die Fachschule für wirtschaftliche Berufe und die Höhere Lehranstalt für wirtschaftliche Berufe, der Verordnung über den Lehrplan der Fachschule für Sozialberufe, der Verordnung über die Lehrpläne der dreijährigen Fachschule und der Höheren Lehranstalt für wirtschaftliche Berufe sowie der Verordnung über die Lehrpläne für Höhere technische und gewerbliche Lehranstalten; Bekanntmachung der Lehrpläne für den Religionsunterricht. Ausgegeben am 17. November 2015.

Fensham, P. J. (2012). "Preparing citizens for a complex world: the grand challenge of teaching socio-scientific issues in science education" in *Science/environment/health: Towards a renewed pedagogy for science education*. eds. A. Zeyer and R. Kyburz-Graber (Dordrecht, New York: Springer), 7–29.

Friedrichsen, P. J., Sadler, T. D., Graham, K., and Brown, P. (2016). Design of a socio-scientific issue curriculum unit: antibiotic resistance, natural selection, and modeling. *Int. J. Des. Learning* 7, 1–18. doi: 10.14434/ijdl.v7i1.19325

Gregory, T. R. (2009). Understanding natural selection: essential concepts and common misconceptions. *Evo. Edu. Outreach* 2, 156–175. doi: 10.1007/s12052-009-0128-1

Hancock, T. S., Friedrichsen, P. J., Kinslow, A. T., and Sadler, T. D. (2019). Selecting socio-scientific issues for teaching. *Sci. Educ.* 28, 639–667. doi: 10.1007/s11191-019-00065-x

Hoffmann, K., Ristl, R., Heschl, L., Stelzer, D., and Maier, M. (2014). Antibiotics and their effects: what do patients know and what is their source of information? *Eur. J. Pub. Health* 24, 502–507. doi: 10.1093/eurpub/ckt112

Hofstein, A., Eilks, I., and Bybee, R. (2011). Societal issues and their importance for contemporary science education—a pedagogical justification and the state-of-the-art in Israel, Germany, and the USA. *Int. J. Sci. Math. Educ.* 9, 1459–1483. doi: 10.1007/s10763-010-9273-9

Howes, E. V., and Cruz, B. C. (2009). Role-playing in science education: an effective strategy for developing multiple perspectives. *J. Elem. Sci. Educ.* 21, 33–46. doi: 10.1007/BF03174721

Khishfe, R., Alshaya, F. S., BouJaoude, S., Mansour, N., and Alrudiyan, K. I. (2017). Students' understandings of nature of science and their arguments in the context of four socio-scientific issues. *Int. J. Sci. Educ.* 39, 299–334. doi: 10.1080/09500693.2017.1280741

Klosterman, M. L., and Sadler, T. D. (2010). Multi-level assessment of scientific content knowledge gains associated with socioscientific issues-based instruction. *Int. J. Sci. Educ.* 32, 1017–1043. doi: 10.1080/09500690902894512

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K. M., Wertheim, H. F. L., Sumpradit, N., et al. (2013). Antibiotic resistance—the need for global solutions. *Lancet Infect. Dis.* 13, 1057–1098. doi: 10.1016/S1473-3099(13)70318-9

Levy, S. B., and Marshall, B. (2004). Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.* 10, S122–S129. doi: 10.1038/nm1145

Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., et al. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* 18, 268–281. doi: 10.1111/j.1469-0691.2011.03570.x

Mayring, P. (2015). *Qualitative Inhaltsanalyse: Grundlagen und Techniken. 12th Edn*. Weinheim and Basel: Beltz Verlag.

McKenney, S. E., and Reeves, T. C. (2019). *Conducting educational design research*. Abingdon, Oxon, New York, NY: Routledge.

McSharry, G., and Jones, S. (2000). Role-play in science teaching and learning. *Sch. Sci. Rev.* 82, 73–82.

Ødegaard, M. (2003). Dramatic science. A critical review of drama in science education. *Stud. Sci. Educ.* 39, 75–101. doi: 10.1080/03057260308560196

O'Neill, J. (2016). Tackling drug-resistance infections globally: final report and recommendations.

Osborne, J. (2010). "Science for citizenship" in *Good practice in science teaching: What research has to say*. eds. J. Osborne and J. Dillon (Maidenhead: Open University Press), 46–67.

Ottander, C., and Ekborg, M. (2012). Students' experience of working with socioscientific issues - a quantitative study in secondary school. *Res. Sci. Educ.* 42, 1147–1163. doi: 10.1007/s11165-011-9238-1

Peel, A., Zangori, L., Friedrichsen, P., Hayes, E., and Sadler, T. (2019). Students' model-based explanations about natural selection and antibiotic resistance through socio-scientific issues-based learning. *Int. J. Sci. Educ.* 41, 510–532. doi: 10.1080/09500693.2018.1564084

Presley, M. A., Sickel, A. J., Muslu, N., Merle-Johnson, D., Witzig, S. B., Izci, K., et al. (2013). A framework for socio-scientific issues based education. *Sci. Educ.* 22, 26–32.

Rädiker, S., and Kuckartz, U. (2019). *Analyse qualitativer Daten mit MAXQDA: Text, audio und video*. Wiesbaden: Springer Fachmedien Wiesbaden.

Rafolt, S., Kapelari, S., and Kremer, K. (2019a). Kritisches Denken im naturwissenschaftlichen Unterricht – Synergiemodell, Problemlage und Desiderata. *Zeitschrift für Didaktik der Naturwissenschaften* 25, 63–75. doi: 10.1007/s40573-019-00092-9

Rafolt, S., Thaler, J., and Kapelari, S. (2019b). "Argumentation about antibiotic resistance in secondary school biology," in *International Conference New Perspectives in Science Education 8th Edition*. ed. Conference proceedings (Bologna: Filodiritto Editore), 559–563.

Roberts, D. A., and Bybee, R. W. (2014). "Scientific literacy, science literacy, and science education" in *Handbook of research on science education*. eds. N. G. Lederman and S. K. Abell (New York: Routledge), 545–558.

Romine, W. L., and Sadler, T. D. (2016). Measuring changes in interest in science and technology at the college level in response to two instructional interventions. *Res. Sci. Educ.* 46, 309–327. doi: 10.1007/s11165-014-9452-8

Romine, W. L., Sadler, T. D., Dauer, J. M., and Kinslow, A. (2020). Measurement of socio-scientific reasoning (SSR) and exploration of SSR as a progression of competencies. *Int. J. Sci. Educ.* 1–22. doi: 10.1080/09500693.2020.1849853 [Epub ahead of print]

Sadler, T. D. (2004). Informal reasoning regarding socioscientific issues: a critical review of research. *J. Res. Sci. Teach.* 41, 513–536. doi: 10.1002/tea.20009

Sadler, T. D. (2009). Situated learning in science education: socio-scientific issues as contexts for practice. *Stud. Sci. Educ.* 45, 1–42. doi: 10.1080/03057260802681839

Sadler, T. D., Barab, S. A., and Scott, B. (2007). What do students gain by engaging in socioscientific inquiry? *Res. Sci. Educ.* 37, 371–391. doi: 10.1007/s11165-006-9030-9

Sadler, T. D., Foulk, J. A., and Friedrichsen, P. J. (2017). Evolution of a model for socio-scientific issue teaching and learning. *Int. J. Educ. Math. Sci. Technol.* 5:75. doi: 10.18404/ijemst.55999

Sadler, T. D., and Zeidler, D. L. (2005). Patterns of informal reasoning in the context of socioscientific decision making. *J. Res. Sci. Teach.* 42, 112–138. doi: 10.1002/tea.20042

Saunders, K. J., and Rennie, L. J. (2013). A pedagogical model for ethical inquiry into socioscientific issues in science. *Res. Sci. Educ.* 43, 253–274. doi: 10.1007/s11165-011-9248-z

Sharland, M., Pulcini, C., Harbarth, S., Zeng, M., Gandra, S., Mathur, S., et al. (2018). Classifying antibiotics in the WHO essential medicines list for optimal use—be AWaRe. *Lancet Infect. Dis.* 18, 18–20. doi: 10.1016/S1473-3099(17)30724-7

Simonneaux, L. (2000). A study of pupils' conceptions and reasoning in connection with "microbes", as a contribution to research in biotechnology education. *Int. J. Sci. Educ.* 22, 619–644. doi: 10.1080/095006900289705

Simonneaux, L. (2001). Role-play or debate to promote students' argumentation and justification on an issue in animal transgenesis. *Int. J. Sci. Educ.* 23, 903–927. doi: 10.1080/09500690010016076

Sjøberg, S., and Schreiner, C. (2012). "Results and perspectives from the rose project" in *Science education research and practice in Europe: Retrospective and prospective*. eds. D. Jorde and J. Dillon (Rotterdam: Sense Publishers), 203–236.

Sørensen, K., van den Broucke, S., Fullam, J., Doyle, G., Pelikan, J., Slonska, Z., et al. (2012). Health literacy and public health: a systematic review and integration of definitions and models. *BMC Public Health* 12:80. doi: 10.1186/1471-2458-12-80

Stake, R. E. (2010). *The art of case study research*. Thousand Oaks, CA: Sage Publications.

Timmis, K., Cavicchioli, R., Garcia, J. L., Nogales, B., Chavarría, M., Stein, L., et al. (2019). The urgent need for microbiology literacy in society. *Environ. Microbiol.* 21, 1513–1528. doi: 10.1111/1462-2920.14611

van Boeckel, T. P., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N. G., et al. (2019). Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science* 365:eaaw1944. doi: 10.1126/science.aaw1944

Van Ments, M. (1999). *The effective use of role-play: Practical techniques for improving learning. 2nd Edn.* London: Kogan Page.

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharm. Ther.* 40, 277–283.

Vrabl, P., and Vrabl, O. (2012). Role-based panel discussions to teach socio-economic consequences of wastewater treatment. *J. Microbiol. Biol. Educ.* 13, 54–56. doi: 10.1128/jmbe.v13i1.349

Waaseth, M., Adan, A., Røen, I. L., Eriksen, K., Stanojevic, T., Halvorsen, K. H., et al. (2019). Knowledge of antibiotics and antibiotic resistance among Norwegian pharmacy customers - a cross-sectional study. *BMC Public Health* 19:66. doi: 10.1186/s12889-019-6409-x

Wassenaar, T. M. (2005). Use of antimicrobial agents in veterinary medicine and implications for human health. *Crit. Rev. Microbiol.* 31, 155–169. doi: 10.1080/10408410591005110

Williams, M. A., Friedrichsen, P. J. D., Sadler, T., and Brown, P. J. (2018). Modeling the emergence of antibiotic resistance in bacterial populations. *Am. Biol. Teach.* 80, 214–220. doi: 10.1525/abt.2018.80.3.214

World Health Organization (2015). Global action plan on antimicrobial resistance. Geneva: World Health Organization.

World Health Organization (2018). Antibiotic resistance - fact sheet.

Zeidler, D. L. (2014). "Socioscientific issues as a curriculum emphasis: theory, research, and practice" in *Handbook of research on science education*. eds. N. G. Lederman and S. K. Abell (New York: Routledge), 697–726.

Zeyer, A., and Dillon, J. (2019). Science|environment|health – the emergence of a new pedagogy of complex living systems. *Discip. Interdscip. Sci. Educ. Res.* 1:263. doi: 10.1186/s43031-019-0014-9

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Combating Antimicrobial Resistance Through Student-Driven Research and Environmental Surveillance

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Emerging resistance to all classes of antimicrobials is one of the defining crises of the 21st century. Many advances in modern medicine, such as routine surgeries, are predicated on sustaining patients with antimicrobials during a period when their immune systems alone cannot clear infection. The development of new antimicrobials has not kept pace with the antimicrobial resistance (AR) threat. AR bacteria have been documented in various environments, such as drinking and surface water, food, sewage, and soil, yet surveillance and sampling has largely been from infected patients. The prevalence and diversity of AR bacteria in the environment, and the risks they pose to humans are not well understood. There is consensus that environmental surveillance is an important first step in forecasting and targeting efforts to prevent spread and transmission of AR microbes. However, efforts to date have been limited. The Prevalence of Antibiotic Resistance in the Environment (PARE) is a classroom-based project that engages students around the globe in systematic environmental AR surveillance with the goal of identifying areas where prevalence is high. The format of PARE, designed as short classroom research modules, lowers common barriers for institutional participation in course-based research. PARE brings real-world microbiology into the classroom by educating students about the pressing public health issue of AR, while empowering them to be partners in the solution. In turn, the PARE project provides impactful data to inform our understanding of the spread of AR in the environment through global real-time surveillance.

Keywords: antimicrobial resistance, antibiotic resistance, environmental surveillance, One Health, science education, citizen science, CURE (course-based undergraduate research experience)

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INTRODUCTION

In October, 2020, the new United States National Action Plan for Combating Antibiotic-Resistant Bacteria 2020–2025 was published (Federal Task Force on Combating Antibiotic-Resistant Bacteria, 2020). This plan builds on the roadmap laid out in 2015, which takes a comprehensive approach to addressing the worldwide problem of antimicrobial resistance (AR).

The approach is rooted in the concept of One Health, which recognizes that human health is inextricably linked to the environment, other animals, and plants (Zinsstag et al., 2011). Since then, coordinated progress has been made in several areas, yet one national goal with enormous untapped potential is that of strengthening national One Health surveillance efforts.

Surveillance of AR in the United States has focused mainly on food testing and epidemiological tracking of clinical infection (Lammie and Highes, 2016). Clinicians and epidemiologists respond primarily to existing threats. Patients present with a resistant infection before epidemiologists are invoked to trace the source and contain the spread. Epidemiological tracking of infectious disease can contain transmission and food testing is a proactive, preventative measure, but more must be done to prevent infections before they arise. As a result of the SARS-CoV-2 pandemic, there is increased awareness of the need to study microbes and transmission patterns in non-clinical settings (i.e., the environment) to prevent the emergence of infectious disease outbreaks.

Antibiotics discharged into the environment provide a selective pressure for survival of microbes harboring AR. Waste streams from livestock, hospitals, households, and industry production of antibiotics can contribute to dissemination of antibiotics and AR into the environment, and AR bacteria and AR genes have been documented in numerous environmental reservoirs (e.g., Aarestrup et al., 1998; Nwosu, 2001; Schwartz et al., 2003; D'Costa et al., 2006; Salyers and Shoemaker, 2006; Lobova et al., 2008; Allen et al., 2010; Institute of Medicine (US) Forum on Microbial Threats, 2010; Wright, 2010; Berglund, 2015; Li et al., 2015). Robust environmental surveillance of water and soil samples has the potential to identify hotspots of AR, which could lead to localized stewardship efforts to contain spread of resistance, possibly preventing AR outbreaks.

Environmental scientists generally conduct in-depth studies to monitor presence and/or abundance of AR genes or levels of culturable AR bacteria in environmental samples from a limited geographic range, but we argue that a more proactive and coordinated approach among clinicians, epidemiologists, and environmental scientists must be taken. For example, there remains a disconnect between the resistance genes surveyed in the environment and those deemed clinically relevant by medical practitioners. Even among scientists, groups rarely use the same sampling schemes, methods, or reporting metrics, making comparisons across studies challenging. We propose that this gap can be addressed, in part, by undergraduates as citizen scientists conducting environmental surveillance to identify environmental hotspots of AR, with findings made available in a database. At the same time, engaging undergraduates in a large research study has the potential to fill an unmet need in undergraduate education – providing more access to authentic research experiences.

Despite the environmental presence of AR bacteria and widespread use of antimicrobials, the public is generally unaware of environmental resistance as a global public health problem (McCullough et al., 2016; Wellcome Trust, 2019). This is compounded by lack of understanding regarding natural selection and how presence of antibiotics leads to selection for AR bacteria. Reduction in use of antimicrobials in agriculture, aquaculture, veterinary, and human medicine are all critical to decrease selective pressure for survival and spread of resistant microbes. One notable example of the power of the informed public is the increase in consumer demand for meat production without antibiotics, which has driven food chains to seek out sources of animals treated with fewer or no antibiotics (Neilsen FreshFacts, 2016; Halloran and Bohne, 2017; Singer et al., 2019). This is significant because interventions that restrict antibiotic use in food animals are tightly associated with a decrease in AR bacteria in these animals (Tang et al., 2017). Collectively, an educated public and the resulting behavioral changes have the power to impact the issue of AR through reducing unnecessary use of antimicrobials.

Currently there is a need for consistent messaging that engages citizens in antibiotic stewardship. Educating the public about the growing AR crisis has been identified in the President's Council of Advisors on Science and Technology (PCAST) *Report on Combating Antibiotic Resistance* as a critical factor for improving antimicrobial stewardship, especially regarding the demand for inappropriate antimicrobial prescriptions (Olson and Riordan, 2012). In addition, many organizations including the [World Health Organization (WHO), 2015], the Wellcome Trust (2019), and the Centers for Disease Control and Prevention (Federal Task Force on Combating Antibiotic-Resistant Bacteria, 2020) have stated that educating the general public is a critical component in the battle against AR. We argue that the classroom is an ideal environment, that of a captive audience, in which to convey consistent, but simple messaging regarding the issue of AR and actions that can be taken by individuals.

COURSE-BASED RESEARCH EXPERIENCES: IMPROVED PEDAGOGY AND INCREASED SCIENTIFIC POTENTIAL

The last 70 years have brought a revolutionary shift in the way we approach teaching laboratory courses. Inquiry-based learning aims to move from the traditional memorization of facts to a process that allows students to "discover" scientific principles through their own experimentation (National Research Council, 1996, 2000; Chiappetta, 2007). Course-based undergraduate research experiences (CUREs) could be considered the next generation of inquiry-based learning. In contrast to inquiry-based instruction, in which the results are generally of limited interest to the broader scientific community, CUREs provide an opportunity for students (typically undergraduates) to collect and analyze data that have potential to lead to new scientific findings of interest beyond the classroom (Lopatto, 2003; Hatfull et al., 2006; Buck et al., 2008; Kloser et al., 2011; Fukami, 2013; Alaimo et al., 2014; Auchincloss et al., 2014; Spell et al., 2014; National Academies of Sciences, Engineering, and Medicine, 2015; Weaver et al., 2020) and are an attempt to scale up the traditional apprentice-style research experiences for undergraduates (Wei and Woodin, 2011;

Olson and Riordan, 2012; National Academies of Sciences, Engineering, and Medicine, 2015). Placing the research experience in the context of a classroom provides opportunities for students who may not otherwise have access to research, such as those attending community colleges or who cannot afford to engage in an unpaid, out-of-class program or internship (Bangera and Brownell, 2014; Hensel and Davidson, 2018).

There is growing recognition that authentic research experiences are valuable to promote desired outcomes in early STEM education, especially retention, improvement in academic achievement, and matriculation into graduate and professional programs. Recently, there has been an explosion in interest in and development of CUREs (e.g., American Association for the Advancement of Science, 2011; Wei and Woodin, 2011; Olson and Riordan, 2012; National Academies of Sciences, Engineering, and Medicine, 2015). In addition to the valuable potential of CUREs to engage and retain STEM students, CUREs have proven effective tools to engage students in tackling scientific challenges that are not well suited to an individual research group, but instead require a large cohort to work en masse.

A key aspect to many successful CUREs is the power of contributing to a collective data set that is available to the larger research community (Hatfull et al., 2006; Shaffer et al., 2010; Jordan et al., 2014; Wiley and Stover, 2014; Leung et al., 2015). Programs with early success in crowd-sourcing student-generated data to tackle big research questions have provided inspiration for scientists in other fields to consider harnessing the power of undergraduates in the classroom to advance science (Elgin et al., 2016, 2017). Students participating in course-based research have contributed to scientific knowledge both through peer-reviewed publications and contributions to databases (e.g., Jordan et al., 2014; Leung et al., 2015; Elgin et al., 2017). The power of student-generated discoveries has been further solidified with the recent publication describing treatment of a cystic fibrosis patient with a combination of engineered, student-derived bacteriophages that killed an infectious strain of *Mycobacterium abscessus* (Dedrick et al., 2019).

THE PREVALENCE OF ANTIBIOTIC RESISTANCE IN THE ENVIRONMENT: A CLASSROOM-BASED ENVIRONMENTAL SURVEILLANCE PROJECT

The Prevalence of Antibiotic Resistance in the Environment (PARE) project is a powerful platform for student learning and scientific discovery that has potential to address the scientific challenges associated with environmental surveillance of AR and to educate tomorrow's decision makers about the public health threat of AR and the biological concept of natural selection. The PARE project is a CURE in which standardized student crowdsourcing data are used to generate a Geographic Information Systems (GIS)-based map of AR data (**Figures 1A,B**). The overarching scientific goal is to identify environmental hotspots of AR that could present risk for human exposure and infection. The educational goal is to make research more equitable for students by creating a scalable, sustainable

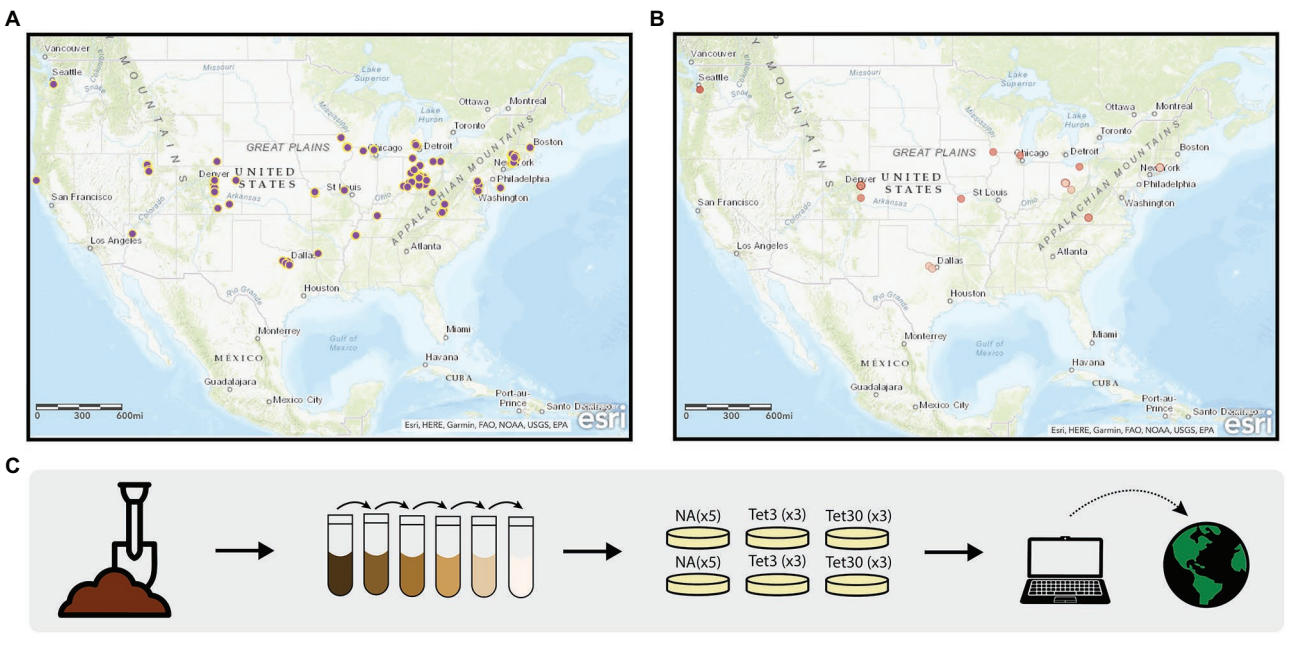

FIGURE 1 | Prevalence of Antibiotic Resistance in the Environment (PARE) Project. The geographic information systems (GIS)-based maps depicting student soil sample collection sites **(A)**, and samples harboring culturable bacteria resistant to 30 μg/ml tetracycline **(B)**. Dark red dots represent samples in which greater than 25% of colony forming units were resistant; light red dots represent less than 25% resistance. **(C)** Outlines the methods used by students to obtain the data shown in the maps. Detailed description of methods available in Genné-Bacon and Bascom-Slack (2018).

research program that can be carried out in classrooms at the undergraduate level, while extending across institution types.

The reach of PARE transcends traditional cohorts of undergraduate biology majors as the curriculum is uniquely situated to engage heterogeneous groups of students beyond the microbiology classroom including non-biology majors and high school students. PARE provides a captivating context for teaching a wide array of core interconnected biological principles (e.g., natural selection, adaptation, mutagenesis, and cell structure/function), while addressing the contemporary public health issue of AR. Students experience and learn about the process of science through the design and execution of experiments, and through grappling with the messiness inherent in authentic experimental data.

In the original, core PARE module (Genné-Bacon and Bascom-Slack, 2018) students collect soil, perform serial dilutions, and plate onto media with or without defined concentrations of tetracycline (**Figure 1C**). Tetracycline was selected for this surveillance project, in part, because it is inexpensive, easy to use and it has a long history of use and study in agriculture (Daghrir and Drogui, 2013; Zhou et al., 2017). Students analyze growth on plates, then calculate and upload the prevalence of tetracycline-resistant colony forming units into a database. Once students upload their PARE results, their prevalence data appears instantly with their soil collection site on an interactive, web-based map. Feedback from participating faculty indicates that a motivator for participating in PARE is the knowledge that students are part of a research community, contributing to a larger body of knowledge (Genné-Bacon et al., 2020).

DATA CURATION

Curation of student-generated data has been a challenge; yet, we have created a new data upload and display system to address many issues that became apparent after the first few years of data collection. Our current system allows display of all soil data collection sites, but blocks display of tetracycline resistance data that do not meet curation criteria. This new GIS-based system was introduced just prior to the onset of the COVID-19 pandemic in which most instructors had to abort their in-person teaching. Nonetheless, this fall, nearly 300 student entries have been recorded. Students upload global positioning system (GPS) metadata captured from a smartphone at the time and place of their sample collection. This eliminates a previous problem of inaccurate location information. Another common observed problem is errors in calculations. For determination of prevalence data, students must count colonies that grow on media with and without tetracycline, and then perform calculations to determine the percent of colonies that are resistant. Analyses of earlier datasets indicated that about 1/3 of entries contained calculation errors. Error types included incorrect use of scientific notation, (e.g., incorrect by an order of magnitude), simple arithmetic errors (e.g., failure to account for dilution factors), and missing data. In our current automated curation, prompts are designed to eliminate scientific notation errors and each step that requires a calculation is calculated automatically based on student colony counts entered. If the computer-generated value does not equal student-generated value, a warning message appears notifying the student and providing opportunity for them to re-check their entries and calculations. Our curation standard for tetracycline resistance data display also requires entry of two duplicate plate sets from the same soil sample. These duplicate counts must not be more than 50% different. These measures have solved many previous problems with the data quality. We cannot, however, verify that students perform accurate colony counts, although two students are instructed to first independently count then compare and arrive at consensus. In addition, depending on the local environment, some dilution sets are simply not countable due to contamination, overgrowth, or undergrowth. To date, of 294 entries, 20% pass our curation standard. While this number is low, we believe, we can reach significant numbers because the growing network of participants implementing PARE includes thousands of students at over 120 undergraduate institutions, approximately 20 high schools, and international partners in France, India, and Botswana. We believe this "first pass" of surveillance complements the rigorous in-depth, but geographically-limited studies being conducted by environmental scientists.

DATA ANALYSIS

Students are asked to form a hypothesis about expected levels of AR in their samples based on collection site characteristics. After resulting AR frequencies are calculated, students first enter data into a shared cloud-based spreadsheet that provides an opportunity to view the classroom data, and to identify and correct errors before database upload. A curated, comprehensive spreadsheet of data is also available on our website for instructors who prefer to have their students work with a larger dataset. In analyses of the data, students are confronted with the messiness of real biological data, which serves as an important learning opportunity and puts them in the shoes of scientists. Students have reported that this reflection helps reinforce the importance of careful attention to methods. Upon reflecting on classroom results, students are asked whether they are consistent with predictions and engage in discussion of potential explanations. Simple analytical activities include determining how best to represent the classroom data and use of *t*-tests to compare average resistance levels at different sites, both of which help build quantitative skills. Some instructors also require students to produce a scientific poster or oral presentation of their results. The student GIS-based database provides enormous potential for data visualization and analysis. Student data can be mapped and analyzed in relation to existing GIS datasets such as land use patterns, pollution/emissions monitoring, and health tracking. A future goal is to augment the curriculum with instructions for students to carry out these analyses.

PARE IS A FLEXIBLE AND INCLUSIVE WAY TO ENGAGE DIVERSE STUDENTS

Approximately one-third of PARE-participating institutions are community colleges. This is notable because there are

known barriers associated with faculty development and implementation of CUREs (Spell et al., 2014; Harris et al., 2015; Shortlidge et al., 2016; Craig, 2017), and data suggest that these tend to be particularly significant for faculty at community colleges (Spell et al., 2014). One way in which PARE lowers barriers for implementation is through its modular design. Since its inception, PARE has partnered with instructors, faculty
experts, and industry to design a suite of classroom research modules, complementary to the core module (**Table 1**).

TABLE 1 | PARE project laboratory module descriptions.

Module name and type	Description	Major skills embedded	Difficulty
Data analysis case study (*Online possible*)	A classroom exercise created with the Great Diseases project, guiding students through a simplified version of a seminal report investigating the outcome of introducing tetracycline use on farms (Jacque et al., 2013).	Data analysis	Introductory level
Molecular case study	A known-outcome case study narrative, created in collaboration with miniPCR™ (https://www.minipcr.com), in which students employ PCR to trace the source of a simulated antimicrobial resistance (AR) outbreak.	PCR Gel electrophoresis	Introductory level
Core module	A culture-based exercise in which soil samples are diluted and plated onto media with and without tetracycline to determine relative prevalence of AR microbes (152).	Serial dilution Plating CFU determination	Introductory or upper level
Virtual colony count (*Online possible*)	An online activity to simulate the Core PARE module.	CFU determination	Introductory or upper level
Identification of Tc^R genes	A non-culture-based activity in which soil DNA is extracted and tested for the presence of two common tetracycline-resistance genes, t*etM* and *tetA* using PCR [created in collaboration with miniPCR™].	DNA extraction PCR Gel electrophoresis	Introductory or upper level
Colony identification	16S rRNA gene sequence analysis is performed on DNA extracted from tetracycline-resistant colonies (isolated in the core module) to make a preliminary phylogenetic assignment.	DNA extraction PCR Gel electrophoresis BLAST search Sequence analysis	Introductory or upper level
Multi-drug resistance testing	Colonies isolated in the core PARE module are tested for resistance to other antimicrobials using a Kirby-Bauer assay.	Culturing Kirby-Bauer testing	Introductory or upper level
Identification of clinically important resistance genes (*in development*)	Soil DNA is extracted and tested for the presence of emergent resistance gene markers (e.g., bla_{NDM1} and *mcr1*).	DNA extraction PCR Gel electrophoresis	Introductory or upper level
Bioinformatics 1 (*Online possible*)	A computer-based sequence analysis activity comparing tetracycline-resistance genes from different bacterial species.	Sequence analysis BLAST search	Introductory or upper level
Bioinformatics 2 (*Online*)	Using on online bioinformatics workflow to search metagenomic soil and water DNA sequence for antibiotic resistance genes.	Interpretation and analysis of bioinformatics data	Upper level
Horizontal transfer	Tests for the ability of the resistance determinant to transfer *via* horizontal gene transfer.	Plasmid extraction Bacterial transformation Plating	Upper level

Modules are generally organized from least to most challenging in terms of technical skills and/or content. There is no specific order in which the modules must be completed. CFU, colony forming units; Tc^R, tetracycline resistance; AR, antimicrobial resistant.

The modules integrate a variety of methods (culture-based and molecular) to assay environmental samples for indications of AR bacteria or associated molecular markers. **Table 1** lists the modules in order of general progression in terms of technical difficulty, time, and reagents required; however, there is no set order in which the modules must be introduced. This flexibility draws instructors from a wide variety of courses and institution types, many of whom have never introduced authentic research into their laboratory courses before. The modular nature of PARE reduces faculty perceived barriers to adopting CUREs by providing faculty with the ability to transition from a traditional laboratory curriculum to a CURE by progressively adding subsequent research modules (Genné-Bacon et al., 2020). It has been documented that, for students, the benefits gained from CUREs increase with time spent immersed within the research project (Shaffer et al., 2014), so it is notable that the majority of PARE instructors transition from implementation of the core module to an expanded experience by their second year of implementation (unpublished results). PARE is continuing to expand its scientific impact through development of additional modules to assess presence of clinically relevant AR genes such as bla_{NDM1}, *mcr1*, and ribosomal methylase-mediated aminoglycoside resistance. Future plans include proactively seeking to detect resistance to newly marketed antimicrobials and those with promise in development.

Many science departments are currently working to integrate a scaffolded CURE experience into the curriculum for undergraduates by offering a progression in which skills or concepts learned in an introductory-level CURE are built upon in a later, upper-level CURE. PARE provides an opportunity for scaffolding of different modules in different courses. In addition, it is complementary to other nationally-disseminated semester-long CUREs such as Tiny Earth/Small World Initiative (Barral et al., 2014, 2016; Hurley et al., 2020), SEA PHAGES (Hatfull et al., 2006; Jordan et al., 2014), and Authentic Research Experience in Microbiology (Muth and McEntee, 2014; Muth and Caplan, 2020). All are focused on some aspect of environmental microbiology, so together they provide ready-to-use options for scaffolding. Tiny Earth provides a particularly appealing complement to PARE due to its focus on discovery of antibiotic-producing microbes from soil samples.

DISCUSSION

A lack of systematic contributions by governmental agencies, non-profits, and research laboratories has left a knowledge deficit that provides an ideal opportunity for PARE to educate students on the problem of environmental resistance and engage them in a research experience with potential for meaningful scientific and societal impact. By engaging a broad constituency of students, including those who will funnel into the healthcare workforce as well as non-STEM sectors of society, PARE directly addresses the need for effective, widely-disseminated messaging to explain the One Health nature of AR and does so in a tangible, personally compelling manner. In addition, the establishment of a standardized environmental surveillance system has the potential to identify hotspots of AR, identify human exposure pathways, monitor for emerging resistant pathogens, and generate data for educational programs to increase public awareness. Increasing understanding of the distribution of AR in their own community not only provides a powerful hook to engage students but also provides an opportunity to enlist them as research partners in a project with broad scientific merit. Students who participate in PARE will further serve as future ambassadors within and outside of healthcare environments, disseminating the critical message that AR is a threat that everyone should not only care about, but one in which they can directly participate in the solution by improving their antimicrobial stewardship.

AUTHOR CONTRIBUTIONS

CB-S wrote the majority of the manuscript with contributions on the environmental perspective from EF and AP and on the clinical perspective from JK, and performed the literature search. JL and AK contributed to sections on the Prevalence of Antibiotic Resistance in the Environment Project and public perceptions. EF created the figure. All authors contributed to the article and approved the submitted version.

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REFERENCES

Aarestrup, F. M., Bager, F., Jensen, N. E., Madsen, M., Meyling, A., and Wegener, H. C. (1998). Surveillance of antimicrobial resistance in bacteria isolated from food animals to antimicrobial growth promoters and related therapeutic agents in Denmark. *APMIS* 106, 606–622. doi: 10.1111/j.1699-0463.1998.tb01391.x

Alaimo, P. J., Langenhan, M., and Suydam, I. T. (2014). Aligning the undergraduate organic laboratory experience with professional work: the centrality of reliable and meaningful data. *J. Chem. Educ.* 91, 2093–2098. doi: 10.1021/ed400510b

Allen, H. K., Donato, J., Wang, H. H., Cloud-Hansen, K. A., Davies, J., and Handelsman, J. (2010). Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.* 8, 251–259. doi: 10.1038/nrmicro2312

American Association for the Advancement of Science (2011). *Vision and change in undergraduate biology education: A call to action*. Washington, DC: AAAS.

Auchincloss, L. C., Laursen, S. L., Branchaw, J. L., Eagan, K., Graham, M., Hanauer, D. I., et al. (2014). Assessment of course-based undergraduate research experiences: a meeting report. *CBE Life Sci. Educ.* 13, 29–40. doi: 10.1187/cbe.14-01-0004

Bangera, G., and Brownell, S. E. (2014). Course-based undergraduate research experiences can make scientific research more inclusive. *CBE Life Sci. Educ.* 13, 602–606. doi: 10.1187/cbe.14-06-0099

Barral, A. M., Makhluf, H., Broderick, N. A., and Kurt, E. L. (2016). The small world initiative™: an innovative crowdsourcing platform for antibiotics. *FASEB J.* 30:665.13. doi: 10.1096/fasebj.30.1_supplement.665.13

Barral, A. M., Makhluf, H., Soneral, P., and Gasper, B. (2014). Small world initiative: crowdsourcing research of new antibiotics to enhance undergraduate biology teaching. *FASEB J.* 28:618.41. doi: 10.1096/fasebj.28.1_supplement.618.41

Berglund, B. (2015). Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. *Infect. Ecol. Epidemiol.* 5:28564. doi: 10.3402/iee.v5.28564

Buck, L. B., Bretz, S. L., and Towns, M. (2008). Characterizing the level of inquiry in the undergraduate laboratory. *J. Coll. Sci. Teach.* 38, 52–58.

Chiappetta, E. L. (2007). *Science as inquiry in the secondary setting*. eds. J. Luft, L. B. Randy and J. Gess-Newsome (Arlington, VA: National Science Teaching Association).

Craig, P. A. (2017). A survey on faculty perspectives on the transition to a biochemistry course-based undergraduate research experience laboratory. *Biochem. Mol. Biol. Educ.* 45, 426–436. doi: 10.1002/bmb.21060

Daghrir, R., and Drogui, P. (2013). Tetracycline antibiotics in the environment: a review. *Environ. Chem. Lett.* 11, 209–227. doi: 10.1007/s10311-013-0404-8

D'Costa, V. M., McGrann, K. M., Hughes, D. W., and Wright, G. D. (2006). Sampling the antibiotic resistome. *Science* 311, 374–377. doi: 10.1126/science.1120800

Dedrick, R. M., Guerrero-Bustamante, C. A., Garlena, R. A., Russell, D. A., Ford, K., Harris, K., et al. (2019). Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant mycobacterium abscessus. *Nat. Med.* 25, 730–733. doi: 10.1038/s41591-019-0437-z

Elgin, S. C. R., Bangera, G., Decatur, S. M., Dolan, E. L., Guertin, L., Newstetter, W. C., et al. (2016). Insights from a convocation: integrating discovery-based research into the undergraduate curriculum. *CBE Life Sci. Educ.* 15:fe2. doi: 10.1187/cbe.16-03-0118

Elgin, S. C. R., Hauser, C., Holzen, T. M., Jones, C., Kleinschmit, A., Leatherman, J., et al. (2017). The GEP: crowd-sourcing big data analysis with undergraduates. *Trends Genet.* 33, 81–85. doi: 10.1016/j.tig.2016.11.004

Federal Task Force on Combating Antibiotic-Resistant Bacteria (2020). National action plan for combatting antibiotic-resistant bacteria 2020–2025.

Fukami, T. (2013). IBI* series winner. Integrating inquiry-based teaching with faculty research. *Science* 339, 1536–1537. doi: 10.1126/science.1229850

Genné-Bacon, E. A., and Bascom-Slack, C. A. (2018). The PARE project: a short course-based research project for national surveillance of antibiotic-resistant microbes in environmental samples. *J. Microbiol. Biol. Educ.* 19:19.3.97. doi: 10.1128/jmbe.v19i3.1603

Genné-Bacon, E. A., Wilks, J., and Bascom-Slack, C. (2020). Uncovering factors influencing instructors' decision process when considering implementation of a course-based research experience. *CBE Life Sci. Educ.* 19:ar13. doi: 10.1187/cbe.19-10-0208

Halloran, J., and Bohne, M. (2017). Chain reaction III: how top restaurants rate on reducing use of antibiotics in their meat supply. Available at: https://advocacy.consumerreports.org/wp-content/uploads/2017/09/ChainReaction3_Report_final.pdf

Harris, A., Babkoor, M., Gu, T., and Kremer, G. E. (2015). "Course-Based Undergraduate Research: A Review of Models and Practices." in *ASME 2015 International design engineering technical conferences and computers and information in engineering conference;* August 2-5, 2015; Boston, MA.

Hatfull, G. F., Pedulla, M. L., Jacobs-Sera, D., Cichon, P. M., Foley, A., Ford, M. E., et al. (2006). Exploring the mycobacteriophage metaproteome: phage genomics as an educational platform. *PLoS Genet.* 2:e92. doi: 10.1371/journal.pgen.0020092

Hensel, N. H., and Davidson, C. N. (2018). *Course-based undergraduate research: Educational equity and high-impact practice*. ed. N. H. Hensel (Washington, DC: Council for Undergraduate Research and Stylus Publishing).

Hurley, A., Chevrette, M. G., Acharya, D. D., Lozano, G. L., Garavito, M., Heinritz, J., et al. (2020). Tiny earth: a big idea for STEM education and antibiotic discovery [Preprint]. *CBE Life Sci. Educ.* doi: 10.20944/preprints202011.0250.v1

Institute of Medicine (US) Forum on Microbial Threats (2010). *Antibiotic resistance: Implications for global health and novel intervention strategies*. Washington, DC: National Academies Press.

Jacque, B., Malanson, K., Bateman, K., Akeson, B., Cail, A., Doss, C., et al. (2013). The great diseases project: a partnership between tufts medical school and the boston public schools. *Acad. Med.* 88, 620–625. doi: 10.1097/ACM.0b013e31828b50fb

Jordan, T. C., Burnett, S. H., Carson, S., Caruso, S. M., Clase, K., DeJong, R. J., et al. (2014). A broadly implementable research course in phage discovery and genomics for first-year undergraduate students. *mBio* 5, e01051–e01113. doi: 10.1128/mBio.01051-13

Kloser, M. J., Brownell, S. E., Chiariello, N. R., and Fukami, T. (2011). Integrating teaching and research in undergraduate biology laboratory education. *PLoS Biol.* 9:e1001174. doi: 10.1371/journal.pbio.1001174

Lammie, S. L., and Highes, J. M. (2016). Antimicrobial resistance, food safety, and one health: the need for convergence. *Annu. Rev. Food Sci. Techol.* 7, 287–312. doi: 10.1146/annurev-food-041715-033251

Leung, W., Shaffer, C. D., Reed, L. K., Smith, S. T., Barshop, W., Dirkes, W., et al. (2015). Drosophila muller f elements maintain a distinct set of genomic properties over 40 million years of evolution. *G3* 5, 719–740. doi: 10.1534/g3.114.015966

Li, B., Yang, Y., Ma, L., Ju, F., Guo, F., Tiedje, J. M., et al. (2015). Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. *ISME J.* 9, 2490–2502. doi: 10.1038/ismej.2015.59

Lobova, T. I., Barkhatov, Y. V., Salamatina, O. V., and Popova, L. Y. (2008). Multiple antibiotic resistance of heterotrophic bacteria in the littoral zone of lake shira as an indicator of human impact on the ecosystem. *Microbiol. Res.* 163, 152–160. doi: 10.1016/j.micres.2006.03.014

Lopatto, D. (2003). The essential features of undergraduate research. *Counc. Undergrad. Res. Q.* 24, 139–142.

McCullough, A. R., Parekh, S., Rathbone, J., Del Mar, C. B., and Hoffmann, T. C. (2016). A systematic review of the public's knowledge and beliefs about antibiotic resistance. *J. Antimicrob. Chemother.* 71, 27–33. doi: 10.1093/jac/dkv310

Muth, T. R., and Caplan, A. J. (2020). Microbiomes for All. *Front. Microbiol.* 11:593472. doi: 10.3389/fmicb.2020.593472

Muth, T. R., and McEntee, C. M. (2014). Undergraduate urban metagenomics research module. *J. Microbiol. Biol. Educ.* 15, 38–40. doi: 10.1128/jmbe.v15i1.645

National Academies of Sciences, Engineering, and Medicine (2015). *Integrating discovery-based research into the undergraduate curriculum: Report of a convocation*. Washington, DC: National Academies Press.

National Research Council (1996). *National science education standards*. Washington, DC: National Academies Press.

National Research Council (2000). *Inquiry and the national science education standards: A guide for teaching and learning*. Washington, DC: The National Academies Press.

Neilsen FreshFacts (2016). Weighing consumers' growing appetite for "clean" meat labeling. Available at: https://www.nielsen.com/us/en/insights/article/2016/weighing-consumers-growing-appetite-for-clean-meat-labeling/ (Accessed May 18, 2020).

Nwosu, V. C. (2001). Antibiotic resistance with particular reference to soil microorganisms. *Res. Microbiol.* 152, 421–430. doi: 10.1016/s0923-2508(01)01215-3

Olson, S., and Riordan, D. G. (2012). "Engage to excel: producing one million additional college graduates with degrees in science, technology, engineering, and mathematics. Report to the President." Executive Office of the President: 130. Available at: http://files.eric.ed.gov/fulltext/ED541511.pdf

Salyers, A., and Shoemaker, N. B. (2006). Reservoirs of antibiotic resistance genes. *Anim. Biotechnol.* 17, 137–146. doi: 10.1080/10495390600957076

Schwartz, T., Kohnen, W., Jansen, B., and Obst, U. (2003). Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiol. Ecol.* 43, 325–335. doi: 10.1111/j.1574-6941.2003.tb01073.x

Shaffer, C. D., Alvarez, C., Bailey, C., Barnard, D., Bhalla, S., Chandrasekaran, C., et al. (2010). The genomics education partnership: successful integration of research into laboratory classes at a diverse group of undergraduate institutions. *CBE Life Sci. Educ.* 9, 55–69. doi: 10.1187/09-11-0087

Shaffer, C. D., Alvarez, C. J., Bednarski, A. E., Dunbar, D., Goodman, A. L., Reinke, C., et al. (2014). A course-based research experience: how benefits change with increased investment in instructional time. *CBE Life Sci. Educ.* 13, 111–130. doi: 10.1187/cbe-13-08-0152

Shortlidge, E. E., Bangera, G., and Brownell, S. E. (2016). Faculty perspectives on developing and teaching course-based undergraduate research experiences. *Bioscience* 66, 54–62. doi: 10.1093/biosci/biv167

Singer, R. S., Porter, L. J., Thomson, D. U., Gage, M., Beaudoin, A., and Wishnie, J. K. (2019). Raising animals without antibiotics: U.S. producer and veterinarian experiences and opinions. *Front. Vet. Sci.* 6:452. doi: 10.3389/fvets.2019.00452

Spell, R. M., Guinan, J. A., Miller, K. R., and Beck, C. W. (2014). Redefining authentic research experiences in introductory biology laboratories and barriers to their implementation. *CBE Life Sci. Educ.* 13, 102–110. doi: 10.1187/cbe.13-08-0169

Tang, K. L., Caffrey, N. P., Nóbrega, D. B., Cork, S. C., Ronksley, P. E., Barkema, H. W., et al. (2017). Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. *Lancet Planet. Health* 1, e316–e327. doi: 10.1016/S2542-5196(17)30141-9

Weaver, G. C., Russell, C. B., and Wink, D. J. (2020). Inquiry-based and research-based laboratory pedagogies in undergraduate science. *Nat. Chem. Biol.* 4, 577–580. doi: 10.1038/nchembio1008-577

Wei, C. A., and Woodin, T. (2011). Undergraduate research experiences in biology: alternatives to the apprenticeship model. *CBE Life Sci. Educ.* 10, 23–31. doi: 10.1187/cbe.11-03-0028

Wellcome Trust (2019). Reframing Resistance 2019. Available at: https://wellcome.ac.uk/reports/reframing-antimicrobial-resistance-antibiotic-resistance (Accessed May 18, 2020).

Wiley, E. A., and Stover, N. A. (2014). Immediate dissemination of student discoveries to a model organism database enhances classroom-based research experiences. *CBE Life Sci. Educ.* 13, 131–138. doi: 10.1187/cbe.13-07-0140

World Health Organization (WHO) (2015). Global action plan on antimicrobial resistance. 2017-04-11 12:45:48 2015. Available at: https://www.who.int/antimicrobial-resistance/publications/global-action-plan/en/ (Accessed May 18, 2020).

Wright, G. D. (2010). Antibiotic resistance in the environment: a link to the clinic? *Curr. Opin. Microbiol.* 13, 589–594. doi: 10.1016/j.mib.2010.08.005

Zhou, Y., Niu, L., Zhu, S., Lu, H., and Liu, W. (2017). Occurrence, abundance, and distribution of sulfonamide and tetracycline resistance genes in agricultural soils across China. *Sci. Total Environ.* 599-600, 1977–1983. doi: 10.1016/j.scitotenv.2017.05.152

Zinsstag, J., Schelling, E., Waltner-Toews, D., and Tanner, M. (2011). From "one medicine" to "one health" and systemic approaches to health and well-being. *Prev. Vet. Med.* 101, 148–156. doi: 10.1016/j.prevetmed.2010.07.003

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Blogging as a Tool for Real-Time Learning in Medical Microbiology

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Blogging is a widely used social medium for storing and sharing information online. Being an attractive online interface, some studies show that education blogging or edublogging might promote more engaged learning. An apathy to contemporary issues related to one's area of study can result in a less knowledgeable student who is less ready for the job industry. To bridge the gap between classroom learning and awareness of emerging issues pertaining to the field of study and potential employment – blogging of ongoing events in a select microbiological field was proposed as a graded semester-long activity called "Disease Tracking." The exercise involved instructing students to choose one infectious disease topic, for which traditional and non-traditional scientific information could be sourced with high frequency over the preceding months. Students were to document new information on the topic as it became available over the term, from reliable information resources. At the end of the term, students presented their work in a "Blog show-off" presentation session. Blog-based learning was found to be an engaging tool that satisfied all criteria under Bloom's taxonomy. Students developed a continued intrigue for the chosen topic and appreciated the diverse fields in which fundamentals of infectious diseases learned in class, could be applied within and outside academia. Students also valued this experience and feedback showed that the freedom to choose their own topic (77%), opportunity to learn more from other students' blogs (77%), less stress as they were not competing on identical topics (73%), a "fun way" to learn (68%), and an opportunity to understand the importance of staying abreast with scientific news (64%) stood out as the chief positive points of the exercise to the students. In view of these benefits, blogs can be used for an immersive, broad learning experience in Microbiology and other fields in which there is likely to be a frequency of new information online.

Keywords: blog, microbiology, e-learning, engaged learning, disease tracking, social media, edublog, infectious disease

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INTRODUCTION

Undergraduate education in long-evolved sciences such as Microbiology (which is the focus of this education research issue) is challenging even for a passionate student, due to the vast content and unfamiliar terminologies (Struwig et al., 2016). Traditional methods of teaching microbiology such as theory and practicals may result solely in the recollection of facts and skills for exams. Activities that could stimulate reasoning (Patil and Karadesai, 2016) or learning

beyond the classroom often take a back-seat due to time constraints. Student-led scientific presentations, journal club discussions, classes, and seminars (Spruijt et al., 2013; Dinkel, 2020) are useful, yet sometimes perceived as boring and heavy tasks (Stuart, 2013). Board games, gaming, and project/case-based learning (Beyfield and Struwig, 2007; Struwig et al., 2014; Mateo and Sevillano, 2018) have been attempted by educators with varying success to reinforce fundamentals. Some educators have even sought the use of social networking such as Whatsapp and Facebook to keep students engaged on case studies, whereas others have reported students to perceive this as sometimes intrusive (Hershkovitz et al., 2019; Van Den Beemt et al., 2020). It is interesting to note that while students are heavily engrossed in social platforms (Abbas et al., 2019), not many have an appetite for trending world events or scientific news (Crane and Cox, 2013; Medrano, 2014). While learning of traditional knowledge and skills are important, students also need to stay abreast with world events pertaining to their chosen discipline of study. Discussion boards on learning management systems (LMS) might be used to promote this. Often, a lack of appeal of the interface, absence of immediate feedback, and poor use of interactive tools by the educator (Ramayah and Lee, 2012) result in lowered students' interest.

Blogging is not new to our tech-savvy undergraduates. They use blogs to record their experiences in life and learning (Zhang and Olfman, 2010); for personal/study reflections, as a repository for learning materials (Yang, 2009; Ginani et al., 2012) and a ready source of information for online communities (Jolly and Matthews, 2017). Why blogs over other social media? Undergraduates perceive blogs as an exciting way to share their "newly learned scholarly knowledge" from a single portal with their networks (Lowe et al., 2016), while also reaping social benefits of networking, such as the gratification in social enhancement, recognition, and appreciation (Srivastava et al., 2019). Educators from a variety of fields have used blogs for collaboration and enhancing learning – for example, marketing blogs, ICT, and industry project development (Luca and McLoughlin, 2005a; Duarte, 2015).

Polytechnic undergraduates in Biomedical Science are required to be job-ready with laboratory skills and strong subject knowledge. If efforts are taken to encourage students into being conversant with scientific affairs beyond the classroom, it may help them make informed choices about their career. In this article, I share my perspective with some examples, as to why blogging on microbiology in the news over a term as a graded exercise, might be an engaging and stimulating learning tool for both the students and the educator.

DISEASE TRACKING FOR EDUBLOGGING IN MICROBIOLOGY

The blogging activity titled "Disease tracking" (DT) was conducted under the medical microbiology module for second year Biomedical science diploma students at Ngee Ann Polytechnic between 2015 and 2018. The module ran for a semester (approximately 4 months). The pre-requisite for this module was a pass in year 1 General Microbiology.

Implementing Blogging and Learning Objectives

The major learning objectives of this exercise was (a) to give students an opportunity to connect foundational knowledge and laboratory techniques learned in the class with current relevant topics published in the field and (b) to ignite an interest in students to look beyond social media and engage in learning from scientific resources.

In week 1, the class collaborated at a tutorial to perform an online search for infectious diseases that appeared frequently in national or international news sites. Examples of these diseases could be frequent articles on an outbreak, clinical trial, antibiotic resistance issues, etc. The topics once finalized, were listed on a shared online document that students used to choose their disease of interest. If this is to be adapted to a larger cohort or undertaken subsequently, then depending on the class size and the number of diseases trending for that span of time, an individual or a team of student blogger(s) could take on a single topic. Students created the blogging site using any of the freely available online blogging tools and the web address was shared to the class using the virtual learning environment. In our case, this was Blackboard. For future cohorts or in different institutions, other Learning Environments would also work.

Expectations

The "Disease Tracking" blog was to be a chronological documentation of a chosen trending infectious disease during the term of study. Articles if sparse could be sourced from 6 months to a year in advance and followed up thereafter. While an introduction was permissible, the blog was not to be a lesson/seminar about the microorganism. It was intended to document every source of information about that infectious disease. The resources could range from but were not limited to – news and scientific news articles; information from Centre for Disease Control (CDC), United States, WHO, Ministry of Health Singapore; and articles from PubMed, research centers, and clinical trial sites. Students were to blog about the articles in their own words, appropriately referencing the sources and avoiding plagiarism. An end-of-term "Blog Show Off" was conducted to promote sharing of the content collected over the term. The whole DT exercise accounted for 15% of the overall grade. Marks were awarded by the lecturer/instructor/teacher, under the following categories: contributions per member, a good distribution of article resources, a proper simplified interpretation, ability to explain the article verbally at the end of presentation (to ensure it was not transcribed without understanding), good presentation skills, and being able to connect back to the fundamentals learned from lectures. A short feedback survey was conducted to understand students' opinions of the blogging exercise. This is discussed in the next section.

DISCUSSION

Some of the examples of the DT blogs prepared by the polytechnic students are tabulated in **Table 1** (provided with permission from students). Within the 12 weeks of a semester, students had to contribute to a minimum of eight posts. The blogs should be seen as a testament to students' abilities to explore subject information and creatively document relevant contemporary events in a study discipline beyond the limits of the curriculum. While this is also possible with journal and seminar preparation and presentation, blogging has the added potential to encourage interaction while it is being developed between blogging groups (Ferdig and Trammel, 2004; Duarte, 2015).

Students' impressions of the positive points in the blogging exercise have been tabulated (**Table 2**). Out of 28 students in the cohort, 22 responders shared their feedback. More than half the respondents agreed that the top reasons why they appreciated the "Disease tracking" blogging exercise was because they got to choose their own topic (77%), they were learning more from other students' blogs (77%), they were not in competition with each other on identical topics (73%), found it a fun way to learn (68%), and understood the importance of staying abreast with scientific news (64%). Less than half the respondents thought that blogging promoted community awareness (46%), or that it was slow paced (36%), or that they learned epidemiology from the exercise (36%). Being acknowledged by fellow readers (27%), being peer reviewed by classmates after presentation or having a blog that was uniquely theirs (27%) mattered less as a positive point. Understandably, only 23% felt more confident through this exercise to research and understand complex papers on the microorganism being blogged (out of increased interest).

TABLE 1 | Examples of students' blogs on disease tracking.

Name of student's blog	Link to blog
Rabies (Low, 2015)	http://rabiesdiseasetracking2015.blogspot.com/
Mersecode (Tan, 2016)	https://mersecode.wordpress.com/
Dengue (Teo, 2017)	https://onceyougodengueyouwontbehealthy.wordpress.com/about/
Human immunodeficiency virus (Song et al., 2018)	https://humanimmunodeficiencyvirusnpbms.wordpress.com/page/2/
Cholera (Ang and Beh, 2018)	https://cholerabms.wordpress.com/

TABLE 2 | Student's impressions on edublogging for learning in the "disease tracking" exercise.

Characteristics of the "disease tracking" blog exercise	Students in agreement with the characteristic *n* = 22 (%)
Freedom to choose student's/team's own topic	17 (77%)
Opportunity to learn more from other students'/teams' blogs	17 (77%)
Less stressful as students were not competing on identical topics	16 (73%)
A "fun way" to learn	15 (68%)
Opportunity to understand the importance of staying abreast with scientific news	14 (64%)
Occasion to learn the importance of blogging in community awareness	10 (46%)
Ownership of a piece of online compilation	9 (41%)
Slow paced nature of the exercise	8 (36%)
Opportunity to learn the epidemiological distributions of disease	8 (36%)
Being acknowledged by fellow readers	6 (27%)
Being peer reviewed by class mates after their presentation	6 (27%)
Presenting a blog that was uniquely their's	6 (27%)
Built confidence to understand complex papers on the microorganism and infectious disease being blogged.	5 (23%)

Understanding the views of students toward this blogging exercise was important and feedback was sought. Written feedback provided by the students also showed that they found this a worthwhile and interesting approach to learning about infectious diseases. Comments included: "very unique educational approach," "less of an obligation, but an interest," "more useful and interesting than doing presentations on PubMed articles," "unique way of presenting information to the class, instead of using PowerPoint," "not done solely just for grades but for learning itself – knowledge I have gained from blogging will be remembered even after this module has ended!," "Having sat through the sharing seasons by my classmates, I feel like I've really taken a lot home, and it felt really rewarding." One respondent felt that "it was quite tough" to incorporate in their busy schedule. The majority (20/22) respondents agreed that they appreciated the importance of the subject better. All respondents recommended this learning method for future cohorts.

As described by other educators (Ferdig and Trammel, 2004; Schroeder et al., 2010), blogging provided a flexibility in teaching and learning and promoted a collaborative knowledge construction. The experience of having a class of engaged listeners through a blog presentation exercise was rewarding. Students were curious about the various snippets of information. The mix of news articles, scientific articles, government policies, international body, and public opinions was able to capture the attention of all listeners. The dynamic variety of colorful blog interfaces when swiped through contributed to visual appeal. It was an eye-opening experience for young polytechnic students to envisage how a fundamental class chapter, for example –antibiotic resistance, had far reaching connections.

As the blog contributed to the students' final grade, the blogging exercise was always completed. Reluctance to blog (Duarte, 2015) by some, led to shabby blogs that were populated close to the deadline. The challenge of discerning fair and equitable work in groupwork (Luca and McLoughlin, 2005b) was not difficult with blogs, as it usually is with other group assignments, as every blog post is accompanied by a time-stamp against the contributor's name. From an education point of view, the exercise of blogging to learn about a trending disease, ticked all the boxes in the Bloom's taxonomy (Bloom, 1956) checklist. The "creativity" and quality

of presentation in the blogs were appealing. Skills in interpreting scientific articles evolved, sometimes with a journalistic flair! Students were able to "evaluate" and "analyze" the extent, burden, and management of disease spread over time, from a wide variety of perspectives (medical, scientific, community, and social). They began to formulate their own views on how the health system was run and began to question policies. It was observed that students were also able to "apply" their understanding garnered from lectures and practicals directly into the "whats and whys" of diagnostic tests, outbreaks, experiments, vaccines, and clinical trials. They were able to "understand" the purpose of learning historical snippets in Microbiology. Students appreciated how knowledge is built up over time/generations and how this knowledge goes into the identification of etiological agents in outbreaks, disease eradication, immunization, the role of community, and the value of strong leadership in such instances. The blog approach caused students to revisit their fundamentals several times, thus helping them to "remember" their basics better.

The role of the lecturer was to commend contributors, stimulate scholarly discussions, and thus gently stimulate more contributions. Students met with additional concepts in immunology, biosecurity, and epidemiology along their learning journey. They were able to appreciate the interdisciplinary approaches taken to study and control infectious diseases.

The advantage of using a blog in microbiology is that there is no dearth of reports of infectious diseases to source from. Blogging about various diseases post-Covid-19 will be important to refocus on the many forgotten but prevalent diseases. Students may also share their blog link in their resumes. Some of the disadvantages in the current experience is that (a) if students did not use their actual names in the blogs, they may be acknowledged only by their user name in citations, (b) blogs cannot be submitted through plagiarism software for checking, (c) in the rare case, a student may decide to take down their blog and it cannot be referenced anymore, and (d) students may or may not decide to continue populating the blog after the course.

A similar blogging exercise can be recommended for any subject, where there is likely to be frequent resources of new information.

CONCLUSION

Blogging about even one disease over one academic term broadened a student's outlook to the myriad scenarios in which microbiology is applied, within and outside academia. This is a good approach to extend the curriculum beyond the classroom. Students become active and independent learners. They see for themselves the various infectious disease problems and gaps in medical management. This spurs them to delve deeper into the subject, ask questions, and become potential problem solvers (Lujan and DiCarlo, 2006) from an early stage in their career.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

The entire work published here-in is the teaching pedagogy designed, executed, and reviewed by the sole author.

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REFERENCES

Abbas, J., Aman, J., Nurunnabi, M., and Bano, S. (2019). The impact of social media on learning behavior for sustainable education: evidence of students from selected universities in Pakistan. *Sustainability* 11:1683. doi: 10.3390/su11061683

Ang, Y. X. A., and Beh, M. Z. (2018). Cholera, Disease Tracking Blog. Available at: https://cholerabms.wordpress.com/ (Accessed January 3, 2021).

Beylefeld, A. A., and Struwig, M. C. (2007). A gaming approach to learning medical microbiology: students' experiences of flow. *Med. Teach.* 29, 933–940. doi: 10.1080/01421590701601550

Bloom, B. S. (1956). *Taxonomy of educational objectives: The classification of educational goals*. New York NY: Longmans, Green.

Crane, J. W., and Cox, J. L. (2013). More than just a lack of knowledge: a discussion of the potential hidden-impact of poor pre-enrolment science background on nursing student success in bioscience subjects. *IJISME* 21, 26–36.

Dinkel, A. B. (2020). Teaching undergraduate immunology using journal club discussions and case studies. *J. Immunol.* 204(Suppl. 1), 222.5.

Duarte, P. (2015). The use of a group blog to actively support learning activities. *Act. Learn. High. Educ.* 16, 103–117. doi: 10.1177/1469787415574051

Ferdig, R. E., and Tramell, K. D. (2004). Content delivery in the 'Blogosphere.' T.H.E. Journal. 31. Available at: https://www.learntechlib.org/p/77152/ (Accessed December 1, 2020).

Ginani, F., Vasconcelos, R. G., and Barboza, C. A. G. (2012). Use of clinical cases in a virtual learning environment as an approach to teaching human embryology. *Int. J. Morphol.* 30, 1395–1398. doi: 10.4067/S0717-95022012000400022

Hershkovitz, A., Abu Elhija, M., and Zedan, D. (2019). WhatsApp is the message: out-of-class communication, student-teacher relationship, and classroom environment. *JITE:Research* 18, 63–95. doi: 10.28945/4183

Jolly, J. L., and Matthews, M. S. (2017). Why we blog: Homeschooling mothers of gifted children. *Roeper Rev.* 39, 112–120. doi: 10.1080/02783193.2017.1289579

Low, E. Q. (2015). Rabies. Available at: http://rabiesdiseasetracking2015.blogspot.com/ (Accessed January 3, 2021).

Lowe, D., Fehlhaber, K., Coyne, J., and Timmer, J. (2016). What drives you to blog? *Cell* 167, 1446–1447. doi: 10.1016/j.cell.2016.11.028

Luca, J., and McLoughlin, C. (2005a). "Supporting collaborative learning with blogs" in *Proceedings of world conference on educational multimedia, hypermedia and telecommunications*, 3711–3714. Available at: https://ro.ecu.edu.au/ecuworks/2637/ (Accessed February 23, 2021).

Luca, J., and McLoughlin, C. (2005b). "Can blogs promote fair and equitable teamwork?" in *22nd Annual Conference of the Australasian Society for Computers in Learning in Tertiary Education "Balance, Fidelity, Mobility: Maintaining the momentum?* Brisban: ASCILITE, 379–386.

Lujan, H. L., and DiCarlo, S. E. (2006). Too much teaching, not enough learning: what is the solution? *Adv. Physiol. Educ.* 30, 17–22. doi: 10.1152/advan.00061.2005

Mateo, E., and Sevillano, E. (2018). Project-based learning methodology in the area of microbiology applied to undergraduate medical research. *FEMS Microbiol. Lett.* 365:fny129. doi: 10.1093/femsle/fny129

Medrano, J. (2014). Students lack knowledge concerning current events. The Crusader. Available at: https://crusadernews.com/2105/opinion/students-lack-knowledge-concerning-current-events/ (Accessed February 23, 2021).

Patil, M. J., and Karadesai, S. G. (2016). To determine the effectiveness of case-based tutorials as compared to traditional tutorials in microbiology. *Natl. J. Integr. Res. Med.* 7, 5–8.

Ramayah, T., and Lee, J. W. C. (2012). System characteristics, satisfaction and e-learning usage: a structural equation model (SEM). *TOJET* 11, 196–206.

Schroeder, A., Minocha, S., and Schneider, C. (2010). The strengths, weaknesses, opportunities and threats of using social software in higher and further education teaching and learning. *J. Comput. Assist. Learn.* 26, 159–174. doi: 10.1111/j.1365-2729.2010.00347.x

Song, S. Y., Lee, Q. E., Ching, N. L., and Tan, J. (2018). Human Immunodeficiency Virus NP BMS. Available at: https://humanimmunodeficiencyvirusnpbms.wordpress.com/page/2/ (Accessed January 3, 2021).

Spruijt, A., Wolfhagen, I., Bok, H., Schuurmans, E., Scherpbier, A., Van Beukelen, P., et al. (2013). Teachers' perceptions of aspects affecting seminar learning: a qualitative study. *BMC Med. Educ.* 3:22. doi: 10.1186/1472-6920-13-22

Srivastava, K., Chaudhury, S., Prakash, J., and Dhamija, S. (2019). Social media and mental health challenges. *Ind. Psychiatry J.* 28, 155–159. doi: 10.4103/ipj.ipj_154_20

Struwig, M. C., Beylefeld, A. A., and Joubert, G. (2014). Learning medical microbiology and infectious diseases by means of a board game: can it work? *Innov. Educ. Teach. Int.* 51, 389–399. doi: 10.1080/14703297.2013.774139

Struwig, M. C., Beylefeld, A. A., and Joubert, G. (2016). Reasons for suboptimal learning in medical microbiology. *Teach. High. Educ.* 21, 590–609. doi: 10.1080/13562517.2016.1163670

Stuart, A. E. (2013). Engaging the audience: developing presentation skills in science students. *J. Undergrad. Neurosci. Educ.* 12, A4–A10.

Tan, S. M. D. (2016). Middle East Respiratory Syndrome coronavirus (MERS-CoV). Available at: https://mersecode.wordpress.com/ (Accessed January 3, 2021).

Teo, S. X. (2017). Dengue Virus. Available at: https://onceyougodengueyouwontbehealthy.wordpress.com/ (Accessed January 3, 2021).

Van Den Beemt, A., Thurlings, M., and Willems, M. (2020). Towards an understanding of social media use in the classroom: a literature review. *Technol. Pedagog. Educ.* 29, 35–55. doi: 10.1080/1475939X.2019.1695657

Yang, S.-H. (2009). Using blogs to enhance critical reflection and community of practice. *J. Educ. Technol. Soc.* 12, 11–21.

Zhang, S., and Olfman, L. (2010). Studios, mini-lectures, project presentations, class blog and wiki: a new approach to teaching web technologies. *JITE:IIP* 9, 187–199. doi: 10.28945/1300

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Real-World Ethical Dilemmas in Laboratory Safety for Microbiology Under-Resourced and Outreach Teaching

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With modernization of safety standards for microbiology outreach teaching laboratories, ethical challenges arise in teaching microbiology for the public good without short-changing students in under-resourced situations, or when institutional support is subpar. Still, educators want students to engage using applied skills for inquiry, research-based microbial learning activities – safely. Following several United States microbial outbreaks, federal investigation traced sources back to teaching laboratories. Policy discussions ensued. The American Society for Microbiology (ASM) Task Force provides recommended but not mandated guidelines; however, guidelines are not amenable by all. Here, a real-world, ethical scenario of a university-level outreach microbiology laboratory course hosted at several locations provides context for under-resourced challenges in safety compliance. In this example of biomedical and public health ethical considerations, upper administration puts the onus on instructors to assure safe labs for their students and the general public. Temporarily hired instructors without curriculum or sufficient institutional support are put in precarious positions with often egregious practices to get the job done. This scenario is examined with different public health ethical frameworks and principles: non-maleficence, beneficence, health maximization, efficiency of policy regulations, respect for institutional and instructor autonomy, justice, and proportionality balancing stakeholder concerns. Sample curricular strategies are employed to mitigate these challenges. Taking a utilitarianism framework of the greatest good for the most benefit, this paper advocates for social justice supporting access to education as a moral duty. Administrations should ensure instructors are supported sufficiently to provide safe, authentic learning experiences. Solutions for under-resourced outreach teaching are needed for public trust.

Keywords: ethics, laboratory, safety, policy, outbreak, risk, curriculum, equity

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INTRODUCTION

Teaching microbiology laboratory courses safely has new meaning and ethical challenges. Even before modern life-altering pandemics begin changing worldviews, raised awareness is needed of ethical safety challenges faced in under-resourced science teaching laboratories. Change away from "normal science" practice creates tensions. Reasoning helps "puzzle-solve" through

crisis (Kuhn, 1962). Exploring ethical dilemmas helps balance competing needs such as increasing stringency of safety in resource-limited settings without limiting learning, sustaining equitable educational opportunities, and negotiating administrative priorities. Every accident or near miss, whether biological or chemical, teaches lessons reminding that safety is integral to science. Worst-case scenario emerging pathogen pandemic planning is attentive to history, changing paradigms in biosafety, social justice, and ethical lenses to mitigate disease. All are trademarks applying public health perspectives (Mack et al., 2007) also necessary in the small-scale educational setting.

Comprehensive, updated biosafety sources (Wooley and Byers, 2017) include specific recommendations addressing the special environment of the college-level teaching laboratory and recognizing burdens and liabilities of instructors from under-resourced settings (Woolverton and Woolverton, 2017). Additional resources and CDC biosafety training modules[1] (**Table 1**) can assist institutional decision-making capabilities to maintain safe standards, even when staff may lack legal protection when some institutions avoid compliance. Generally, biosafety officers assist instructors to assure safe student instruction environments. Without institutional support and oversight, sometimes the instructor alone makes the decision to use practices beyond biosafety level (BSL)1 criteria, conducted on a standard laboratory table with minimal personal protective equipment (PPE), e.g., optional gloves and eye protection. Some practices, e.g., discouraged isolation from environmental sources, could isolate BSL2 organisms and pose infectious risk. Even well-equipped laboratories working within established laboratory safety practices have risk (Hayden, 2011) as seen in several multi-state outbreaks[2,3,4] of a pathogenic strain of *Salmonella* Typhimurium originating in clinical and teaching laboratories.

In response, an American Society for Microbiology (ASM) task force drafted and revised guidelines (Emmert, 2013; Woolverton, 2013; Byrd et al., 2019). An updated addendum,[5] clarifies use of risk group RG1 organisms, and better accounts for the range of emergent issues in teaching facilities and laboratory practices. Guidelines are recommended, but not mandated. However, as enhanced safety guidelines evolve, they do not fully account for additional burdens that arise in under-resourced institutions. Assumptions of how microbiology is supposed to function often fail to include alternate viewpoints and practices in under-resourced settings.

Guidelines are assumed to be beneficial. Beneficence promotes a safety-ethics culture to prevent hazards, near-misses, or unreported incidents regardless of the science, size of laboratory or setting (Hill, 2016). Even small hazards in a teaching laboratory with untrained, introductory-level students may pose risk for undocumented laboratory-acquired infection (LAIs) (Carlberg and Yeaman, 2006). Harding and Byers (2006) review the epidemiological approach of distribution in populations and LAIs from research, clinical, and teaching. Outbreaks from teaching laboratories are low, but not systematically monitored or reported. Impacts include host susceptibility, behavioral factors, and the environment. Despite benefits, guidelines can also cause harm. Maleficence can occur when they are misunderstood, ill-fitting for the environment, or mandates produce unintended consequences.

Real-world biomedical challenges and public health ethical dilemmas are not new for under-resourced institutions with faculty struggling to provide microbiology laboratory courses safely. What appears non-standard for the mainstream is standard in another, termed "under-resourced" or "outreach" for the purpose of this article includes formal learning in different modalities: distance education, online and hybrid courses using do-it-yourself (DIY) at-home kits, citizen science, and laboratory courses hosted at different sites *via* a traveling lab bus. Assumptions begin with faculty having solid foundational understanding and respect for microorganisms and safety. Recognizing "one-size-fits-all" standards are not feasible, faculty and institutions adhere to a "good-faith effort" (Woolverton and Woolverton, 2017). However, what happens if these assumptions are not met, when a sole safety-trained scientist is alone pushing for reform, or when the upper administration is more concerned about the financial bottom line and appearance of effort without the true fidelity and commitment? These questions of safety and social justice in education are best addressed applying a public health ethics equity approach.

Under-resourced outreach teaching example University X provides a real-world scenario (**Table 1**). Challenges and failure to meet laboratory safety guidelines and other dilemmas are examined using a novel approach applying public health ethical analysis. Social justice issues surrounding the development and implementation of guidelines raises potential harm if mandated too harshly or when under-resourced institutions fail to respond well. Public health policymaking applies several frameworks. Schröder-Bäck et al. (2014) outlines seven principles to explore cases such as under-resourced University X: non-maleficence, beneficence, health maximization, efficiency, respect for autonomy, justice, and proportionality. Here, to assure that biosafety restrictions do not limit learning science in an unjust manner, this analysis raises awareness of the minority voice of under-resourced institutions.

NON-MALEFICENCE IN BIOSAFETY GUIDELINE COMPLIANCE AND ACCEPTABLE RISK IN CHANGING PARADIGMS

Changing paradigms increase conflict by altering what constitutes acceptable risk, biosafety measures, and abilities to comply. The basis of bioethics and public health ethics is the Hippocratic oath "*primum nil nocere*," taken as the first principle of non-maleficence and "do not harm." There is a duty to educate as well as protect health. Lack of compliance to safety policy guidelines can harm, as can mandating too harshly.

[1]https://www.cdc.gov/training/QuickLearns/biosafety/
[2]https://www.cdc.gov/salmonella/2011/lab-exposure-1-17-2012.html
[3]https://www.cdc.gov/salmonella/typhimurium-labs-06-14/index.html
[4]https://www.cdc.gov/salmonella/typhimurium-07-17/index.html
[5]https://asm.org/Guideline/ASM-Guidelines-for-Biosafety-in-Teaching-Laborator

TABLE 1 | Sample University X under-resourced teaching laboratory scenario.

Real-world scenarios	Challenge	Under-resourced responses, solutions, and persistent remaining challenges
Under-resourced example		
• University X, a United States-based, originally brick-and-mortar institution teaches university courses at globally-located sites in host countries and online. • Serves a diversity of underrepresented and lower SES students, providing a valuable education and next step. • Provides STEM core courses for credentials.	• Unique settings present challenges for different science laboratory courses – particularly microbiology. • Mission to keep tuition low creates culture of scarcity. • Need to assure courses are sustained for STEM pipeline discoveries, innovations, health and economic growth with adequate numbers of research and public health professionals.	• Unique solutions to daily challenges are sought. • Some solutions employed are not within guidelines. • Less costly supervisory roles lack safety-trained leadership. • Mandates and guidelines may be ignored if not enforceable, yet puts greater moral burden and culpability on instructors.
Failure to meet compliance		
• United States Occupational Safety and Health Act of 1970 (OSH Act, 2011). • Institution provided laboratory courses practices common to most institutions until 1990 OSHA Laboratory Standard regulations for safety education requires protection for employees from hazards causing serious harm. • Regulations specify Chemical Hygiene Officer, written Chemical Hygiene Plans, and generally a biosafety officer.	• Institutional non-compliance. • Inability to keep up with safety changes without allocated resources and leadership.	• Administration seeks steps to compliance: - OSHA Occupational Safety and Health Administration (2011). • Administration hires support staff. • Provide faculty training and safety officer assistance. • Accessible resources: - updated (Wooley and Byers, 2017) built on prior safety guidelines (Fleming and Hunt, 2006). - Special teaching laboratory environment (Woolverton and Woolverton, 2017). - ASM resources.[8] - International recommendations World Health Organization (WHO, 2004). - Online resources (Barber and Stark, 2015). - Biosafety Level (BSL) criteria for risk assessment learned through Center for Disease Control (CDC) training.[1] - National Research Council guidelines for chemicals (National Research Council, 2011, 2014). • Policy change can support faculty with safe compliant, low-cost, curricula meeting educational competency needs and retaining courses.
• University X neither kept up, nor meets OSHA regulations; faculty and OSHA-trained students recognize breaches; faculty risk-takers have higher tolerance for poor compliance; students grateful for education are less likely to report complaints.	• Exempted or non-compliant from mandates e.g., education contracted on military bases with exemptions, online education, or by negligence.	
• Administration lacking trained guidance makes top-down, unilateral decisions directly impacting educational safety standards: cuts online microbiology laboratory courses, allows other coursework to continue without safety oversight, and remains in violation of biomedical ethical standards in many host countries abroad for non-compliant storage, transport, access, and use of chemical and biological materials. • Institutional policies to inform faculty and provide training support lack leadership.	• Administrative decision-making by Dean and Academic Coordinators under the approval of the top administration is flawed without consultation with experts and solutions.	
• Those with more knowledge hold greater responsibility and culpability if an accidental exposure and/or outbreak occurred without power to address the issues. • Internal and external whistleblowing is a response as a consequence of a failed action.	• Responsibility, culpability, and autonomy are complex.	
Under-resourced settings		
• Laboratory courses are held wherever space is available. Faculty drive to each site with supplies to set up the "lab" before class (in variable settings), teach lecture and lab, break it down, clean, dispose waste to move back to a storage area or their own homes. • An advertisement depicting a remote area boldly claims "*Where others see this, we see a classroom*." Science knows no bounds. Even non-traditional microbiology learning can take the form of a traveling lab bus for access. • Without safe disposal, microbial waste is sewaged, dissected formalin-preserved specimens (from Anatomy course) put in woodchippers to "hide" waste in trash.	• Outreach sites struggle with additional challenges to meet guideline compliance. • With no storage in shared spaces, microbial plates are put in incubation tubs labeled "do not touch" left at "room temp" in a public office space, or personal vehicle. • Diverse locations lack space for storage, sample incubation, or cleaning. Without areas to autoclave waste, everything must be cleared for immediate disposal or safe transport.	• Meet logistical challenges through community effort to collectively design and provide safe curricula for different outreach settings not meeting guidelines. • Attempt to collectively generate ideas. - One-day experiments without stored incubation. - Pressure cooker instead of autoclave or bring waste to hospital.

(Continued)

TABLE 1 | Continued

Real-world scenarios	Challenge	Under-resourced responses, solutions, and persistent remaining challenges
Under-resourced faculty preparation		
• Faculty are temporarily hired, may have limited training in microbiology, little practical skill, nor aware of ASM resources or safety guidelines. • With lack of guidance, institution remains unaware of current standards. • Administration puts the onus on instructors to assure safe laboratory courses with assumptions that temporarily hired instructors have basic knowledge to teach the course. • Those coming from well-supported institutions struggle with low resources and do not recognize unintended consequences of stringent guidelines. • Faculty relying on older traditional methods are not familiar with current guidelines. • Under-resourced faculty experience isolation. • Without a supported lab manual curriculum, or institutional safety officer, faculty are sole decision-makers. • Sole safety-aware science instructor (microbiologist) may become the lone voice seeking institutional change. • Frustration mounts when the institution lacks safety officer to support faculty or designate faculty member as biosafety leader without training or credentials. • Hierarchy designates safety officers to bear the burden of liability, but places a higher burden on the instructor resulting in fears of liability, culpability, or guilt if an accident or near miss occurs. • Embarrassment identifying with an institution blinded more by finances than by safety. • Might not publish without compliance or fear of public exposure.	• Lacking preparation and with a knowledge gap in where to find resources, guidelines, or curricula, faculty rely on their prior knowledge; older methods of isolation from environmental sources are practiced. • Without stock cultures, yogurt provides the easiest source, but new temporary faculty step into the position (sometimes with less than 2 weeks to prepare) with the same challenge not knowing where to begin. • Isolation keeps under-resourced faculty from finding assistance. • Fear hampers reaching out *via* listservs and putting self and institution at risk of further culpability. • With questionable institutional practices, not only is safety a concern but also culpability when compliance is not met. • When the institution lacking safety measures does not require (or even allow) faculty publishing, then raises an ethical concern of justice.	• Trained mentor or administrator provides orientation packet of resources with safety training and curricular ideas attempts to address gaps faculty face. However, with set syllabi and curricula, this results in the risk of lost autonomy in curricular decisions. • Community of educators provides support.
Under-resourced curriculum support		
• Without a curriculum, a biosafety officer or suggestions to get started, faculty relies on what is "on hand" and think outside the box. • Piecemeal lab kits putting faculty in precarious positions often with egregious practices to get the job done. • With sparsely allocated resources of funds, time, and staff to minimally address chemicals, PPE and biological waste disposal, the pros and cons of stringent guidelines yield different forms of harm.	• Challenges meeting ASM curricular learning outcomes[6]: "Ways to properly prepare and view specimens for examination using microscopy, use pure culture and selective techniques to enrich for and isolate microorganisms and methods to identify microorganisms."	• Those with knowledge can use open-source resources indicate curricula for under-resourced needs: - ASM guidelines.[6] - ASM resource curricula.[8] - Course Source.[9]
Under-resourced supplies		
• Instructors not receiving laboratory supplies, purchase materials, and transport laboratory grade chemicals in personal vehicles, sometimes across international borders. • Lacking supplies, storage, safety training, etc., a laboratory kit pieced together supports several standard lab exercises. Instructors acquaint themselves with available supplies. Since no laboratory manual curriculum is available, they make due to provide a laboratory course.	• Logistics of transport, shipping, labeling, and storing supplies must be considered at every level for safety beyond home regulations. • Lacking standardized training, supplies, and curricular resources, a trained safety officer or administrator could provide safe solutions. • Creative solutions, such as portable eyewashes, hand wash stations, and proactive thinking attempt to meet safety needs.	• Faculty reimbursement for supplies: grocery store low-budget material purchases rather than maintaining a chemical cabinet. • Develop or identify safe curricula suggestions and common supplies to meet competencies. • Identify standard curricula proper disposal methods or alternative solutions. • Provide safe ideas for drop off facilities at hospitals or veterinary disposal for lab animals (Anatomy course).

(Continued)

TABLE 1 | Continued

Real-world scenarios	Challenge	Under-resourced responses, solutions, and persistent remaining challenges
• Faculty bringing sheep brains from farms (for Anatomy course use) were discouraged from doing (potential prion disease). • Faculty working jointly in clinics bringing clinical isolates from hospital patient cases were discouraged from doing so (pathogens).	• Autonomous faculty may use practices with risk guidelines help assist change.	
Risk of mandated guideline (cancelled course)		
• Paradigms changed to legally required (1990 OSHA Laboratory Standard). University X did not meet compliance as one indicator of deficiency and poor staff support. • Following 2012 ASM Task Force recommended guidelines, knowledgeable faculty advocated addressing risk concerns. • Raising awareness of breach in compliance internally results in some measures to attempt compliance, but not always satisfactorily: - Rather than comply, disposal of all chemicals at sites results in lower resources. - Shipping instead of driving chemicals across borders with improper transportation in personal vehicles affects laboratory exercises. - Online microbiology laboratory course cancellation and lost course opportunity. - Institutional discussions to cancel microbiology altogether considered. - Attempts to provide a safe curriculum still continue discouraged environmental isolation microbiology practices as if status quo.	• Mandated-policy loopholes and recommended, non-enforceable guidelines meant administration choses only to address some safety needs. • Responsiveness varies with exemptions and no watchdog. • Centralized command spans large area with communication and leadership gaps. • Increasing faculty concern for their safety and culpability with no trained safety officer. • Raising awareness of guidelines increases faculty awareness of their risk. • Kneejerk response to safety concerns results in lost course, jobs, and student access with greater impacts for low SES under-represented students.	• Do not assume even legal mandates are enforced. • Use bottom-up community discussions involving faculty knowledge, practices, and addition training and curricula. • Suggest safely bought, stored, and transported materials. • Develop curricular ideas to sustain at-risk courses and promote social justice. • Even if under-resourced or low support, provide educators safety training and curricula resources for their own autonomous decision. • Before course cancellation, community solutions may help.
Potential student and staff infections		
• An introductory undergraduate University X student learned aseptic technique in microbiology laboratory training. He steadfastly isolated and identified his unknown from the "handwashing lab" paper towel environmental source. On Monday returning from a weekend of eating out at a buffet and profuse illness, he also identified his unknown as the toxin-forming *Bacillus cereus* that can cause the same symptoms, and questioned his source of illness that could have spread to his newborn or immunocompromised partner. • Contrast this with an isolate originating from soil in a historically endemic area for *B. anthracis* known to persist for decades. A staff member untrained in safety cleans up plates from environmental soil samples and is unknowingly exposed. The new faculty member not knowing the risks seeks information to determine the risk of anthrax. • Educator fears resurrecting the dormant endospore of the organism responsible for the Henle-Koch Postulates and Germ Theory of Disease. *Bacillus anthracis* endospores are resistant to heat, produce anthrax toxin, and are still found globally in soil and zoonosis with potential for outbreak (Koch, 1877; Evans, 1976; Meselson et al., 1994).	• Without safety protocols, there is no protocol for reporting or diagnosis. • Student, staff, and public at risk from laboratory practice. • Commonly found *Bacillus cereus* endospores easily isolated from paper towels or tabletops can cause a toxin-induced food poisoning (Dohmae et al., 2008; Gendron et al., 2012). • Dosage impacts virulence. • Several *Bacillus* species found environmentally are valuable non-pathogenic surrogate models (Greenberg et al., 2010), but there is no guarantee students encounter only non-pathogenic varieties. • Accidental isolation appears lower risk since laborious process requires particular growth media and techniques, but not a guarantee of safety as passage in an animal host to become pathogenic (Dragon and Rennie, 1995, 2001; Cieslak and Eitzen, 1999; Saile and Koehler, 2006).	• Safety training begins with students to consider risks of isolating a pure culture. • Staff training is also needed if potential contact. • These events support following the guidelines for the special environment teaching lab resource (Woolverton and Woolverton, 2017). • The risks from two different species vary but with educator concerns for student and staff safety, a risk assessment would rule out these sources from the environment for isolation.

(Continued)

TABLE 1 | Continued

Real-world scenarios	Challenge	Under-resourced responses, solutions, and persistent remaining challenges
Dramatic examples persuading change		
• Guideline illustration "Do not subculture unknown microbes isolated from the environment because they may be organisms that require BSL2 practices and facilities" (Emmert, 2013). • Faculty resist change citing outreach resembles traditional settings. Pasteur and Koch had risks as they searched for the cause of disease in their laboratories, kitchens, or the back room of a house. • Faculty cite tradition having long taught "isolation of unknowns" as students swabbed from various sources: spices, soil, bathroom sinks, and toilets, or their own computers, cell phones, and hands. • Potential pathogens are commonly found: *Salmonella* on sprouts, toxin-producing *Escherichia coli* on hamburger or water in the common coliform lab exercise, *Staphylococcus aureus* on skin or nasal passages, some with MRSA drug resistance. • Online microbiology faculty believe students practicing aseptic technique at home safely. • Risk and ethical dilemmas increase with human body source rectal and throat swabs, or animal sources from farm or pets. • Faculty believe their farm animal sources to be free of disease. • Clinical faculty believe their patient isolates to be safely contained. • Each outbreak publicly raises awareness of emerging pathogens from animal sources and points to changing paradigms of outbreak from SARS, MERS, and COVID-19 (Salata et al., 2019; Guarner, 2020).	• Wine, beer, and dairy simultaneously studied with causes of outbreaks (Blevins and Bronze, 2010). • Overly dramatic and timely example provides a valid warning: - Virus causing COVID-19 with its probable animal origin has other possible domesticated drivers promoting its spread (Saegerman et al., 2020). - Faculty imagine having an outbreak originate from a swabbed isolate through zoonosis from a student's pet (Halsby et al., 2014). - Multistate *Salmonella* outbreaks originating from pet hedgehogs (Anderson et al., 2017) - Ethical discussions of benefits vs. risks in artwork permeated with pathogenic bacteria (Fawcett and Dumitriu, 2018) - Nationwide outbreaks from United States microbiology teaching laboratories transported by cell phones traced back by federal investigations even from well-equipped laboratories within established laboratory safety practices.[2,3,4]	• First step is prevention of hazards in the environment (Gostin and Wiley, 2016). • As risks are identified, then faculty more open to change if a balance is found and the imposed mandates protect the public but with fairness and fairly distributed resources. • With cost-effective, evidence-based promotion of health, then even if mandates are paternalistic but provide adaptable safe curricula, then all stakeholders could benefit.
Alternate methods for environmental isolation		
• The 2019 Guideline addendum[5] still warns against culturing from the environment; if done, then sealing and not further sampling or handling once microbes have been purified and propagated (Byrd et al., 2019). • With absolutely no supplies (sometimes because shipments do not arrive), faculty create a microbiology lab but always seeking new safe methods that achieve learning outcomes. They try to fit the guidelines. • Grow microbes on potatoes "sterilized" in a microwaved dish and inoculated with "sterilized" Q-tips and observe fungal growth on potatoes sealed to prevent exposure without opening or disturbing. • Koch's postulates are modeled with milk and yogurt cultures. • Baker's yeast shows size comparisons or microscopic eukaryotes with smaller prokaryotes and demonstrates a positive gram stain alongside fermenting bacteria in yogurt or sourdough.	• Low-resourced teaching choices are made to find the balance between the need for the content in science education to promote scientific literacy or new discovery in inquiry-based learning with what can be done safely. • One argument is traditional microbiology laboratory exercises isolating from environmental, their own human body, or other animal sources provide ease of source materials and low cost when it is not possible to order and maintain stock cultures. • Without accessible alternate methods, the updated guidelines pose risks, and ethical decisions with updated guidelines must be made. • Continued need for developed and shared curricula, free public health workshops, and recommendations for early reporting if anything amiss.	• Update curricula: addressing cell phones, not taking laboratory notebooks home in backpacks, training for donning PPE, isolation, and social distancing. • Determine alternate methods to properly prepare and view specimens for examination using microscopy, use pure culture and selective techniques to enrich for and isolate microorganisms and methods to identify microorganisms. • Risk is mitigated if students do not open the plates once obtained, or if pure cultures are obtained by isolating from safer, non-pathogenic sources such as yogurt. • If selective media is not available, obtain unused, expired plates from hospital clinical laboratory. • Use of alternative sliced potatoes lack firm colony formation, but useful for growth; gelatin lacks higher temperature incubation, but useful for aseptic technique; colored photographs and visual libraries useful for demonstrations and critical thinking exercises but lack hands on.

The framework of Kass (2001) asks the overall public health goals, program (guidelines) effectiveness, and potential burdens. If the goals, such as safety, cannot be implemented fairly to mitigate burdens, then we as a society collectively decide procedurally what should and should not be done to protect the health and maintain the education of communities. Bernheim et al. (2009) promotes procedural justice through ethical reflection of all affected groups being part of the decision-making process. The ASM community endeavors to duly discuss biosafety in educational teaching laboratories. Publication in journals requires explanation of adherence to safety guidelines; however, even this rigor can exclude. Burdens faculty face creates tensions when facing the moral code that they "ought" to meet guidelines – particularly when the institution fails them. When more voices are heard, then a balance for the under-resourced can procedurally be sought.

The framework of Baum et al. (2007, 2009) helps manage tensions in daily work by asking how the program guidelines advance wellbeing and respond to the needs of the community. Resolution of conflicts is determined by how burdens created by mandates (or even recommended guidelines) can be minimized through improved alternative approaches for fairness in equity and wellbeing. Rather than theoretically assuming safe practices, feasibility is considered in the daily practice.

The safety guidelines suggest risk assessment to prevent harm, e.g., student mishap, exposure, or a bigger contagion. The principle of non-maleficence is balanced with degrees of harm that would give the greater benefits. Guidelines are acceptable when other harms they create are limited. This utility by Bentham's measure of wellbeing evokes the doctrine of utilitarianism of providing the greatest good for the greatest number (Bentham, 1781, 1996). However, utilitarianism is flawed since consequences are not predictable. In applying consequentialist theory, the actions of utility deemed most correct is one that provides the most benefit gain for the majority (Roberts and Reich, 2002). Utilitarianism is challenged by social justice and the needs of the minority if utility is only increased for the majority.

When all voices are not heard equally, policy guidelines can result in harmful unintended consequences. Harm results if fear or administrative ignorance results in course cancellations. Without alternative approaches, under-resourced educators face burdens of teaching laboratory courses with inadequate safety vs. offering no course at all.

University X (**Table 1**), dedicated to low tuition for lower socioeconomic (SES) students, limits funding resources, temporarily hires faculty, lacks lab manual curricula, and lacks institutional safety officers. Overwhelmed by deficient support, faculty rely on piecemeal lab kits "on hand" and think outside the box to teach authentic science, often putting faculty in precarious positions with egregious practices to get the job done. The slippery slope begins when faculty trying to encourage greater student engagement use "let's give it a shot" attitude and "let's try it and see" to justify their choices (Tippins et al., 1993). Educators knowledgeable of the risks advocate for support, sometimes as the lone voice seeking institutional change. The unguided administration can balk and retaliate resulting in microbiology laboratory course cancellations and in doing so, denying access to education and science literacy. Ethical frameworks applied by the scientific community can help address the underlying moral conflict of stringent biosafety guidelines causing harm.

BENEFICENCE, HEALTH MAXIMIZATION, AND EFFICIENCY

The crux lies in balancing acceptable "tradeoffs" between non-maleficence degrees of harm and the second principle of beneficence, the obligation to produce benefit. To weigh the beneficence of guidelines, risks are ascertained with the broader view of the third principle, health maximization including the greater population. Risk assessment of small-scale threats are similar to the larger scale National Response Framework emergency management cycle: prevention of hazards, risk identified, and fairness imposing mandates to protect (Gostin, 2000a,b,c, 2010; Gostin and Powers, 2006; Gostin and Wiley, 2016). Public health law ties mandates to different degrees of enforceable governmental regulation and even non-mandated, non-enforced guidelines imply obligation through semantics (Harmon, 2016). The moral burden put upon an instructor, whether sufficiently supported or not, and the guilt and culpability that would be incurred if an accident occurred increases the ethical dilemma.

Guidelines elicit a public health benefit to student populations. If pressure from restrictive measures threatens the course loss, then a counter benefit is for the greater public good with an obligation to provide laboratory education supporting science literacy and the welfare of others. Science literacy and microbial appreciation are increasingly important at every level of our global society to understand how scientific understanding changes through evidence. We need laboratory courses for a full science curriculum for our future scientists and health care workers, as well as policy makers, agencies, and general population (Timmis et al., 2019). In sustaining more science courses, then the fourth principle, efficiency, promotes greater impacts. By assuring evidence-based, cost-effective, safe practices for under-resourced education, then science literacy is maintained without short-changing learning even with subpar institutional support.

These trade-offs are exemplified as University X struggles with more stringent guidelines when applying skills of the standard "isolation of unknown" as one form of discovery meeting ASM curricular learning outcomes.[6] Outside of the standard of practice, instructors still resort to isolation practices not consistent with guidelines. Lacking stock cultures, students swab different environmental, their own human body, or other animal sources to isolate unknown microorganisms. Microbes grow, students streak to isolate pure culture colonies, and stain to identify. Risk increases working with environmental cultures

[6]https://asm.org/Guideline/ASM-Curriculum-Guidelines-for-Undergraduate-Microb

if a pathogen is propagated in pure culture; yet, reliance on these traditional practices provides ease of source materials and low cost when it is difficult to order and maintain stock cultures. Alternate methods are sought within guideline recommendations (**Table 1**). Course-based undergraduate research experiences (CURES) for institutions that desire applications of real-world, authentic research experiences but may lack research infrastructure have additional needs (Alkaher and Dolan, 2014; Auchincloss et al., 2014; Davis et al., 2017). Some have expanded Tiny Earth soil projects for broader educational applications with adapted protocols for genomic identification and pivot to online with the pandemic[7] (Basalla et al., 2020).

For new educators, even well trained from R1 research institutions, temporary visiting professor, or adjuncts, the shift to low-resourced education can be daunting with subpar institutional support, proving difficult to navigate and ensure safe, meaningful curricula. Institutions of any type might struggle when modalities change due to life-altering pandemics. When courses globally shift online, many instructors face new challenges teaching laboratory courses authentically and safely, but more so if institutional resources and instructor preparedness are limited (Hodges et al., 2020; Procko et al., 2020; Rapanta et al., 2020). Even providing critical thinking curricula can be a challenge when resources are limited (Aparna et al., 2020, this issue; Song et al., 2016). Hierarchy designates safety officers bear the burden of liability, but places a higher burden of culpability on the instructor. Frustration mounts when the institution lacks a safety officer and inability to publish with embarrassing institutional breaches.

When instructor practices are noncompliant with guidelines, they may need deeper investigation to determine risks of students potentially isolating a pathogenic microorganism from environmental sources (**Table 1**). Even the easily adapted "handwashing" or "disinfectant" labs with resistant bacteria found in soil and paper towels are not without risk since any immunocompromised situation, even pregnancy, increases risks. Although most human infectious disease pandemics originate from cross-species transmission, these are rare in a teaching laboratory (Hughes et al., 2010). However, several dramatic and timely examples provide a valid warning (**Table 1**). Any anomaly away from "normal science" pushes the paradigm change.

RESPECT FOR AUTONOMY VS. TOP-DOWN "PATERNALISTIC" MANDATES

Within public health frameworks, paternalistic guidelines mandated from the top-down are contrasted with the fifth principle of respect for autonomy as a moral consideration (Childress et al., 2002). Academic decision-making by institutions and instructors to comply with guidelines, or not, is a moral choice if guidelines limit individual liberties, or academic freedom. A disadvantage to autonomy is the ethical burden of poor compliance: the student choosing not to comply, the instructor desiring autonomy in teaching strategies, or the administration failing to adequately provide support.

Some educators find themselves advocating for policy changes at their own institutions but in precarious positions of power dynamics. If educators advocate too firmly or take a whistleblowing approach, then courses could be canceled and jobs lost. University X with an inability to comply (or administrative choice not to allocate funds) may result in a knee jerk reaction to cancel microbiology courses putting future generations at risk with the increasing fear of science and lack of knowledge that protects us all and limits justice.

JUSTICE FOR EQUITABLE ACCESS

Baylis et al. (2008) highlight frameworks that focus on a social justice approach for the common good. This calls upon relational autonomy, solidarity of common interests rather than "us and them," and justice in the fairness of how decisions are made. With the consequentialist approach, respect for individual stakeholder interests is unbalanced. Taking the deontological, duty-based approach, policies holding social justice take priority for the most good. Supporting faculty in being able to adhere to a duty-based approach applies normative ethical theory; a moral code determines if an action is right or wrong under a set of rules (Bellefleur and Keeling, 2016). However, if the rules only assume adequate resources, then this adds burden to the duty under-resourced educator's bear.

When the need to follow updated safety guidelines poses threats to course cancellations, then the under-resourced institutions are at further risk. To increase social justice, education needs to reach beyond those in college who cannot afford education by expanding the greater good through promoting science literacy. A hidden part of the unintended consequences of this dilemma is that more of those who come from lower SES attend these under-resourced colleges (Engberg and Allen, 2011). Engaged learning such as laboratory courses offered at community colleges, minority-serving institutions, and from educational opportunities provided through the United States military contracts at home and abroad along with other outreach settings is valuable. Engagement matters in student retention and success (Kuh and Pascarella, 2004; Pike, 2004; Kuh et al., 2006), so this potential cancellation of courses presents a social justice dilemma by limiting science courses that keep low SES students on the trajectory toward graduation, further degree completion, and next steps. Despite increasing college enrollments for underrepresented ethnic minorities, the trends for educational attainment of science, technology, engineering, and mathematics (STEM) degrees and overall graduation within 6 years show disparities (de Brey et al., 2019; McFarland et al., 2019; Cahalan et al., 2020). It is fundamental that resources are attainable, guidelines are equitable, and stigma is limited.

Microbiology fluency, laboratory practices, and equitable access to these skills must be met for mastery of concepts through equitable opportunities and completion (National Academies Science Engineering Medicine, 2018). The vision that all students who desire access to learning, should obtain

[7]https://tinyearth.wisc.edu/

it is addressed through the Partnership for Undergraduate Life Sciences Education (PULSE) with rubrics to measure and promote Vision and Change (Brancaccio-Taras et al., 2016). Although this is useful to achieve goals of modern competencies (Woodin et al., 2010) across different institution types, some under-resourced institutions such as University X are missed in this revolution and feel the gap.

CONCLUSION WITH PROPORTIONALITY OF INDIVIDUAL FREEDOM WITH PUBLIC GOOD

By applying public health frameworks, the primary goal is to mitigate harm in populations: harm from risk to health and from the unintended consequences of policies. The seventh and last principle of proportionality balances that the probable benefits of guidelines for the public good outweigh the infringement on the few. While considering non-maleficence, then compliance to guidelines could promote equitable safety, but harm could occur if equitable educational opportunity is lost. Guidelines with implied instructor culpability, or mandated with severe restrictions and without solutions, create inequitable gaps.

A solution of least infringement and equity to ensure stringent guidelines do not compromise student learning is to provide specifically written safe curricula to aid compliance. There are alternate methods to achieve learning outcomes and still promote compliance (**Table 1**). When advocating risk assessment, guidelines specifically recommend ideas to address the challenges under-resourced faculty face. This is attempted through contributions from diverse institutional types compiling creative ideas for non-traditional settings in open-source-shared curricula, i.e., ASM's Microbe Library[8] or Course Source.[9] Broader dissemination to institutions is a proposed solution if educators themselves lack knowledge. This practice of under-resourced shared teaching ideas helps mitigate harm of excess burden placed on the instructor to meet guidelines when lacking institutional support. Reaching out through the community using an evaluated process with confidentiality assures all legitimate stakeholder voices are involved in providing equitable opportunities and protection for those from underserved populations most at risk of under-resourced courses. In this manner, justice would distribute an equitable, compliant, curriculum, while burdening all to comply.

Educators collectively assure healthy conditions in microbiology teaching laboratory courses philosophically through normative ethics: educators "ought" to be informed by updated guidelines, "ought" not to continue methods with higher risk, and institutions "ought" to provide faculty the curricular and biosafety officer support needed for optimal safety within constraints. Although utilitarianism allows protection and greater accessibility, we must still rely on morality as defined by social contract theorists to apply social justice frameworks for the underserved. Sometimes individual observation when seeing something amiss begs a moral duty to make the correction. Rather than waiting for adverse events, some try whistleblowing in the case of non-compliance or institutional protection. A notable voice, Dr. Li, first signaling the COVID-19 outbreak stated "I think a healthy society should not have just one voice" (Green, 2020). It is for this reason that the voices of the under-resourced must be heard in providing solutions.

[8]https://www.asmscience.org/VisualLibrary
[9]https://www.coursesource.org/

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REFERENCES

Alkaher, I., and Dolan, E. L. (2014). "Integrating research into undergraduate courses" in *Research based undergraduate science teaching*. eds. D. W. Sunal, C. S. Sunal, E. L. Wright, C. L. Mason and D. Zollman (Charlotte, NC: Information Age Publishing, Inc.), 403–434.

Anderson, T. C., Marsden-Haug, N., Morris, J. F., Culpepper, W., Bessette, N., Adams, J. K., et al. (2017). Multistate outbreak of human *Salmonella* Typhimurium infections linked to pet hedgehogs–United States, 2011–2013. *Zoonoses Public Health* 64, 290–298. doi: 10.1111/zph.12310

Aparna, Y., Anuradha, K., Jyothi, C., Manjari, K. S., and Challa, A. K. (2020). Bringing real-world microbiology experiences to undergraduate students in resource-limited environments. *Front. Microbiol.* 11:589405. doi: 10.3389/fmicb.2020.589405

Auchincloss, L. C., Laursen, S. L., Branchaw, J. L., Eagan, K., Graham, M., Hanauer, D. I., et al. (2014). Assessment of course-based undergraduate research experiences: a meeting report. *CBE Life Sci. Educ.* 13, 29–40. doi: 10.1187/cbe.14-01-0004

Barber, N. C., and Stark, L. A. (2015). Online resources for understanding outbreaks and infectious diseases. *CBE Life Sci. Educ.* 14:fe1. doi: 10.1187/cbe.14-12-0221

Basalla, J., Harris, R., Burgess, E., Zeedyk, N., and Wildschutte, H. (2020). Expanding Tiny Earth to genomics: a bioinformatics approach for an undergraduate class to characterize antagonistic strains. *FEMS Microbiol. Lett.* 367:fnaa018. doi: 10.1093/femsle/fnaa018

Baum, N. M., Gollust, S. E., Goold, S. D., and Jacobson, P. D. (2007). Looking ahead: addressing ethical challenges in public health practice. *J. Law Med. Ethics* 35, 657–667. doi: 10.1111/j.1748-720X.2007.00188.x

Baum, N. M., Gollust, S. E., Goold, S. D., and Jacobson, P. D. (2009). Ethical issues in public health practice in Michigan. *Am. J. Public Health* 99, 369–374. doi: 10.2105/AJPH.2008.137588

Baylis, F., Kenny, N. P., and Sherwin, S. (2008). A relational account of public health ethics. *Public Health Ethics* 1, 196–209. doi: 10.1093/phe/phn025

Bellefleur, O., and Keeling, M. (2016). *Utilitarianism in public health*. Montréal, Québec: National Collaborating Centre for Healthy Public Policy.

Bentham, J. (1781). *An introduction to the principles of morals and legislation*.

Bentham, J. (1996). *An introduction to the principles of morals and legislation: The collected works of Jeremy Bentham*. UK: Oxford University Press.

Bernheim, R. G., Nieburg, P., and Bonnie, R. J. (2009). "Ethics and the practice of public health" in *Law in public health practice. 2nd Edn*. eds. R. A. Goodman, R. E. Hoffman, W. Lopez, G. W. Matthews, M. A. Rothstein and K. L. Foster. 2nd ed (Oxford: Oxford University Press).

Blevins, S. M., and Bronze, M. S. (2010). Robert Koch and the 'golden age' of bacteriology. *Int. J. Infect. Dis.* 14, e744–e751. doi: 10.1016/j.ijid.2009.12.003

Brancaccio-Taras, L., Pape-Lindstrom, P., Peteroy-Kelly, M., Aguirre, K., Awong-Taylor, J., Balser, T., et al. (2016). The PULSE vision & change rubrics, version 1.0: a valid and equitable tool to measure transformation of life sciences departments at all institution types. *CBE Life Sci. Educ.* 15:ar60. doi: 10.1187/cbe.15-12-0260

Byrd, J. J., Emmert, E., Maxwell, R., and Townsend, H. (2019). Guidelines for biosafety in teaching laboratories version 2.0: a revised and updated manual for 2019. *J. Microbiol. Biol. Educ.* 20:20.3.57. doi: 10.1128/jmbe.v20i3.1975

Cahalan, M. W., Perna, L. W., Addison, M., Murray, C., Patel, P. R., and Jiang, N. (2020). Indicators of higher education equity in the United States: 2020 historical trend report. Pell institute for the study of opportunity in higher education.

Carlberg, D. M., and Yeaman, M. R. (2006). "Biosafety in the teaching laboratory" in *Biological safety: Principles and practices*. eds. D. O. Fleming and D. L. Hunt (Hoboken, NJ, USA: John Wiley & Sons, Inc.), 531–549.

Childress, J. F., Faden, R. R., Gaare, R. D., Gostin, L. O., Kahn, J., Bonnie, R. J., et al. (2002). Public health ethics: mapping the terrain. *J. Law Med. Ethics* 30, 170–178. doi: 10.1111/j.1748-720X.2002.tb00384.x

Cieslak, T. J., and Eitzen, E. M. Jr. (1999). Clinical and epidemiologic principles of anthrax. *Emerg. Infect. Dis.* 54, 552–555. doi: 10.3201/eid0504.990418

Davis, E., Sloan, T., Aurelius, K., Barbour, A., Bodey, E., Clark, B., et al. (2017). Antibiotic discovery throughout the Small World Initiative: a molecular strategy to identify biosynthetic gene clusters involved in antagonistic activity. *Microbiologyopen* 6:e00435. doi: 10.1002/mbo3.435

de Brey, C., Musu, L., McFarland, J., Wilkinson-Flicker, S., Diliberti, M., Zhang, A., et al. (2019). Status and trends in the education of racial and ethnic groups 2018 (NCES 2019-038). U.S. Department of Education. Washington, DC: National Center for Education Statistics.

Dohmae, S., Okubo, T., Higuchi, W., Takano, T., Isobe, H., Baranovich, T., et al. (2008). *Bacillus cereus* nosocomial infection from reused towels in Japan. *J. Hosp. Infect.* 69, 361–367. doi: 10.1016/j.jhin.2008.04.014

Dragon, D. C., and Rennie, R. P. (1995). The ecology of anthrax spores: tough but not invincible. *Can. Vet. J.* 36, 295–301.

Dragon, D. C., and Rennie, R. P. (2001). Evaluation of spore extraction and purification methods for selective recovery of viable *Bacillus anthracis* spores. *Lett. Appl. Microbiol.* 33, 100–105. doi: 10.1046/j.1472-765x.2001.00966.x

Emmert, E. A. (2013). Biosafety guidelines for handling microorganisms in the teaching laboratory: development and rationale. *J. Microbiol. Biol. Educ.* 14, 78–83. doi: 10.1128/jmbe.v14i1.531

Engberg, M. E., and Allen, D. J. (2011). Uncontrolled destinies: improving opportunity for low-income students in American higher education. *Res. High. Educ.* 52, 786–807. doi: 10.1007/s11162-011-9222-7

Evans, A. S. (1976). Causation and disease: the Henle-Koch postulates revisited. *Yale J. Biol. Med.* 49, 175–195.

Fawcett, N. J., and Dumitriu, A. (2018). Bacteria on display—can we, and should we? Artistically exploring the ethics of public engagement with science in microbiology. *FEMS Microbiol. Lett.* 365:fny101. doi: 10.1093/femsle/fny101

Fleming, D. O., and Hunt, D. L. (2006). *Biological safety: Principles and practices. 4th Edn.* Washington, DC*:* ASM Press.

Gendron, L. M., Trudel, L., Moineau, S., and Duchaine, C. (2012). Evaluation of bacterial contaminants found on unused paper towels and possible postcontamination after hand washing: a pilot study. *Am. J. Infect. Control* 40, e5–e9. doi: 10.1016/j.ajic.2011.07.007

Gostin, L. O. (2000a). Public health law in a new century: part I: law as a tool to advance the community's health. *JAMA* 283, 2837–2841. doi: 10.1001/jama.283.21.2837

Gostin, L. O. (2000b). Public health law in a new century: part II: public health powers and limits. *JAMA* 283, 2979–2984. doi: 10.1001/jama.283.22.2979

Gostin, L. O. (2000c). Public health law in a new century: part III: public health regulation: a systematic evaluation. *JAMA* 283, 3118–3122. doi: 10.1001/jama.283.23.3118

Gostin, L. O. (2010). *Public health law and ethics: A reader. Vol. 4*. Berkeley, CA: University of California Press.

Gostin, L., and Powers, M. (2006). What does social justice require for the public's health? Public health ethics and policy imperatives. *Health Aff.* 25, 1053–1060. doi: 10.1377/hlthaff.25.4.1053

Gostin, L. O., and Wiley, L. F. (2016). "Public health emergency preparedness terrorism pandemics and disaster" in *Public health law: Power, duty, restraint.* Berkeley, California: University of California Press, 391–694.

Green, A. (2020). Li Wenliang. *Lancet* 395:682. doi: 10.1016/S0140-6736(20)30382-2

Greenberg, D. L., Busch, J. D., Keim, P., and Wagner, D. M. (2010). Identifying experimental surrogates for *Bacillus anthracis* spores: a review. *Investig. Genet.* 1:4. doi: 10.1186/2041-2223-1-4

Guarner, J. (2020). Three emerging coronaviruses in two decades: the story of SARS, MERS, and now COVID-19. *Am. J. Clin. Pathol.* 153, 420–421. doi: 10.1093/ajcp/aqaa029

Halsby, K. D., Walsh, A. L., Campbell, C., Hewitt, K., and Morgan, D. (2014). Healthy animals, healthy people: zoonosis risk from animal contact in pet shops, a systematic review of the literature. *PLoS One* 9:e89309. doi: 10.1371/journal.pone.0089309

Harding, A. L., and Byers, K. B. (2006). "Epidemiology of laboratory-associated infections" in *Biological safety: Principles and practices*. eds. D. O. Fleming and D. L. Hunt (Hoboken, NJ, USA: John Wiley & Sons, Inc.), 53–77.

Harmon, A. G. (2016). "Should" or "must": distinguishing mandates from guidelines in tort claims contexts. *W. Va. L Rev.* 118:1007.

Hayden, E. C. (2011). *Salmonella* hits US teaching labs. *Nature* 473:132. doi: 10.1038/473132a

Hill, R. H. (2016). The impact of OSHA's laboratory standard on undergraduate safety education. *J. Chem. Health Saf.* 23, 12–17. doi: 10.1016/j.jchas.2015.10.017

Hodges, C., Moore, S., Lockee, B., Trust, T., and Bond, A. (2020). The difference between emergency remote teaching and online learning. Educause review, 27.

Hughes, J. M., Wilson, M. E., Pike, B. L., Saylors, K. E., Fair, J. N., LeBreton, M., et al. (2010). The origin and prevention of pandemics. *Clin. Infect. Dis.* 50, 1636–1640. doi: 10.1086/652860

Kass, N. E. (2001). An ethics framework for public health. *Am. J. Public Health* 91, 1776–1782. doi: 10.2105/AJPH.91.11.1776

Koch, R. (1877). Untersuchungen uber bakterien V. Die aetiologie der milzbrand-krankheit, begrunder auf die entwicklungegeschichte *Bacillus anthracis*. *Beit. Biol. Pflanzen* 2, 277–310.

Kuh, G. D., Kinzie, J. L., Buckley, J. A., Bridges, B. K., and Hayek, J. C. (2006). *What matters to student success: A review of the literature. Vol. 8*. Washington, DC: National Postsecondary Education Cooperative.

Kuh, G. D., and Pascarella, E. T. (2004). What does institutional selectivity tell us about educational quality? *Change Magaz. High. Learn.* 36, 52–59. doi: 10.1080/00091380409604986

Kuhn, T. S. (1962). *The structure of scientific revolutions*. Chicago: University of Chicago Press.

Mack, A., Choffnes, E. R., Sparling, P. F., Hamburg, M. A., and Lemon, S. M. (2007). *Ethical and legal considerations in mitigating pandemic disease: Workshop summary*. Washington, DC: National Academies Press.

McFarland, J., Hussar, B., Zhang, J., Wang, X., Wang, K., Hein, S., et al. (2019). *The condition of education 2019. NCES 2019-144*. Washington, DC: National Center for Education Statistics.

Meselson, M., Guillemin, J., Hugh-Jones, M., Langmuir, A., Popova, I., Shelokov, A., et al. (1994). The Sverdlovsk anthrax outbreak of 1979. *Science* 266, 1202–1208. doi: 10.1126/science.7973702

National Academies of Sciences, Engineering, and Medicine (2018). *Indicators for monitoring undergraduate STEM education*. Washington, DC: The National Academies Press.

National Research Council (2011). *Prudent practices in the laboratory: Handling and management of chemical hazards, updated version*. Washington, DC: National Academies Press.

National Research Council (2014). *Safe science: Promoting a culture of safety in academic chemical research*. Washington, DC: National Academies Press.

Occupational Safety and Health Administration (2011). Laboratory safety guidance. Occupational Safety and Health Administration US OSHA.

Pike, G. R. (2004). Measuring quality: a comparison of US news rankings and NSSE benchmarks. *Res. High. Educ.* 45, 193–208. doi: 10.1023/B:RIHE.0000015695.84207.44

Procko, K., Bell, J. K., Benore, M. A., Booth, R. E., Moore, V. D. G., Dries, D. R., et al. (2020). Moving biochemistry and molecular biology courses online in times of disruption: recommended practices and resources-a collaboration with the faculty community and ASBMB. *Biochem. Educ.* 48, 421–427. doi: 10.1002/bmb.21354

Rapanta, C., Botturi, L., Goodyear, P., Guàrdia, L., and Koole, M. (2020). Online university teaching during and after the Covid-19 crisis: refocusing teacher presence and learning activity. *Postdig. Sci. Educ.* 1, 923–945. doi: 10.1007/s42438-020-00155-y

Roberts, M. J., and Reich, M. R. (2002). Ethical analysis in public health. *Lancet* 359, 1055–1059. doi: 10.1016/S0140-6736(02)08097-2

Saegerman, C., Bianchini, J., Snoeck, C. J., Moreno, A., Chiapponi, C., Zohari, S., et al. (2020). First expert elicitation of knowledge on drivers of emergence of influenza D in Europe. *Transbound. Emerg. Dis.* 1–11. doi: 10.1111/tbed.13938

Saile, E., and Koehler, T. M. (2006). *Bacillus anthracis* multiplication, persistence, and genetic exchange in the rhizosphere of grass plants. *Appl. Environ. Microbiol.* 72, 3168–3174. doi: 10.1128/AEM.72.5.3168-3174.2006

Salata, C., Calistri, A., Parolin, C., and Palù, G. (2019). Coronaviruses: a paradigm of new emerging zoonotic diseases. *Pathog. Dis.* 77:ftaa006. doi: 10.1093/femspd/ftaa006

Schröder-Bäck, P., Duncan, P., Sherlaw, W., Brall, C., and Czabanowska, K. (2014). Teaching seven principles for public health ethics: towards a curriculum for a short course on ethics in public health programmes. *BMC Med. Ethics* 15:73. doi: 10.1186/1472-6939-15-73

Song, D., Karimi, A., and Kim, P. (2015). A remotely operated science experiment framework for under-resourced schools. *Interact. Learn. Environ.* 24, 1706–1724. doi: 10.1080/10494820.2015.1041407

Timmis, K., Cavicchioli, R., Garcia, J. L., Nogales, B., Chavarría, M., Stein, L., et al. (2019). The urgent need for microbiology literacy in society. *Environ. Microbiol.* 21, 1513–1528. doi: 10.1111/1462-2920.14611

Tippins, D. J., Tobin, K. G., and Hook, K. (2007). Dealing with dilemmas of laboratory science: making sense of safety from a constructivist perspective. *Int. J. Sci. Educ.* 15, 45–59. doi: 10.1080/0950069930150104

WHO (2004). *Laboratory biosafety manual*. Geneva, Switzerland: World Health Organization.

Woodin, T., Carter, V. C., and Fletcher, L. (2010). Vision and change in biology undergraduate education, a call for action—initial responses. *CBE Life Sci. Educ.* 9, 71–73. doi: 10.1187/cbe.10-03-0044

Wooley, D. P., and Byers, K. B. (2017). *Biological safety: Principles and practices*. Hoboken, NJ, USA: John Wiley & Sons, Inc.

Woolverton, C. J. (2013). JMBE supports 20 years of teaching excellence at ASMCUE and offers ASM laboratory safety guidelines. *J. Microbiol. Biol. Educ.* 14:1. doi: 10.1128/jmbe.v14i1.591

Woolverton, C. J., and Woolverton, A. K. (2017). "Biological safety and security in teaching laboratories" in *Biological safety: Principles and practices*. eds. D. P. Wooley and K. B. Byers (Hoboken, NJ, USA: John Wiley & Sons, Inc.), 563–584.

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Genomics of Environmental *Salmonella*: Engaging Students in the Microbiology and Bioinformatics of Foodborne Pathogens

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We have developed and implemented an undergraduate microbiology course in which students isolate, characterize, and perform whole genome assembly and analysis of *Salmonella enterica* from stream sediments and poultry litter. In the development of the course and over three semesters, successive teams of undergraduate students collected field samples and performed enrichment and isolation techniques specific for the detection of *S. enterica*. Eighty-eight strains were confirmed using standard microbiological methods and PCR of the *invA* gene. The isolates' genomes were Illumina-sequenced by the Center for Food Safety and Applied Nutrition at the FDA and the Virginia state Division of Consolidated Laboratory Services as part of the GenomeTrakr program. Students used GalaxyTrakr and other web- and non-web-based platforms and tools to perform quality control on raw and assembled sequence data, assemble, and annotate genomes, identify antimicrobial resistance and virulence genes, putative plasmids, and other mobile genetic elements. Strains with putative plasmid-borne antimicrobial resistance genes were further sequenced by students in our research lab using the Oxford Nanopore MinION™ platform. Strains of *Salmonella* that were isolated include human infectious serotypes such as Typhimurium and Infantis. Over 31 of the isolates possessed antibiotic resistance genes, some of which were located on large, multidrug resistance plasmids. Plasmid pHJ-38, identified in a Typhimurium isolate, is an apparently self-transmissible 183 kb IncA/C2 plasmid that possesses multiple antimicrobial resistance and heavy-metal resistance genes. Plasmid pFHS-02, identified in an Infantis isolate, is an apparently self-transmissible 303 kb IncF1B plasmid that also possesses numerous heavy-metal and antimicrobial resistance genes. Using direct and indirect measures to assess student outcomes, results indicate that course participation contributed to cognitive gains in relevant content knowledge and research skills such as field sampling, molecular techniques, and computational analysis. Furthermore, participants self-reported a deeper interest in scientific research and careers as well as psychosocial outcomes (e.g., sense of belonging and self-efficacy) commonly associated with student success and persistence in STEM. Overall, this course provided

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a powerful combination of field, wet lab, and computational biology experiences for students, while also providing data potentially useful in pathogen surveillance, epidemiological tracking, and for the further study of environmental reservoirs of *S. enterica*.

Keywords: *Salmonella*, course-based undergraduate research, pathogen reservoirs, bioinformatics, pathogen surveillance, environmental pathogens, public health microbiology, genomic epidemiology

INTRODUCTION

Bacterial genomic epidemiology – the use of genomics-based methods to aid in the epidemiological investigation of communicable diseases – has become an important new tool in the hands of public health laboratories tasked with tracking pathogen outbreaks (Deng et al., 2016; Armstrong et al., 2019). Most outbreaks have been studied retrospectively due to the costs and time involved in analyzing pathogens using pulsed-field gel electrophoresis. However, the introduction of massively parallel sequencing technologies – along with the application of bioinformatics algorithms for assembly, typing, annotation, and phylogenetic analysis – have begun to enable the real-time tracking of outbreaks for infection control and prevention. Whole genome sequencing (WGS) can help public health scientists better understand the origins and dynamics of the outbreak itself (Tang et al., 2017; Armstrong et al., 2019), while providing important information about outbreak strains – such as their serotype, antibiotic susceptibility, potential virulence factors, and toxins – in a single, *in silico* assay (Nadon et al., 2017).

Non-typhoidal *Salmonella enterica* (NTS) are the leading cause of foodborne illness in the United States, and one of the main causes of gastrointestinal disease globally. Worldwide, there are 1.3 billion reported cases of gastroenteritis, 16 million cases of typhoid fever, and 3 million deaths annually attributed to all *S. enterica* infections (Bhunia, 2018), with ca. 535,000 attributed to NTS in 2017 (Parisi et al., 2020). NTS are spread via the fecal-oral route and transmitted via contaminated foods (e.g., poultry, beef, dairy, and eggs), water, and direct animal contact (Silva et al., 2014; Bhunia, 2018). Non-typhoidal *S. enterica* typically causes self-limiting gastroenteritis; however, it can cause invasive disease under certain circumstances (Crump et al., 2015). Effective real-time tracking of new outbreaks requires a large database of *Salmonella* from clinical, food, animal, and environmental sources. As of this writing, over 286,000 draft and complete genomes of *Salmonella* have been sequenced. However, to date only ca. 18,608 are identified as having been isolated from environmental sources such as freshwaters and soils[1]. Irrigation waters are potential sources of *Salmonella* outbreaks in foods (Bell et al., 2015; Liu et al., 2018), and recreational waters may also act as sources for infections in both humans and animals (Levantesi et al., 2012). Unlike other enteric bacteria like *E. coli*, *S. enterica* has high survival rates in aquatic systems and soils, can persist in poultry houses for over a year, and is generally more resistant to environmental fluctuations. It has been postulated that its long-term survival in such secondary habitats facilitates passage to its next host (Winfield and Groisman, 2003). In order to rapidly source human infections in the event of an outbreak, potential environmental reservoirs as well as clinical sources of *S. enterica* need to be monitored.

In keeping with national reform calls in science, technology, engineering, and mathematics (STEM) education to engage all undergraduates in the authentic practice of scientific discovery (National Research Council (US) Committee on Undergraduate Biology Education to Prepare Research Scientists for the 21st Century, 2003; American Association for the Advancement of Science, 2011; National Academies of Sciences, Engineering, and Medicine, 2017) we designed a semester-long course-based undergraduate research experience (CURE) in which upper-division undergraduate students are immersed in the isolation, characterization, and genomic analysis of *S. enterica* isolated from the local environment. As students gain access to more advanced research practices as part of their normal laboratory coursework, a CURE such as this offers students an equitable and inclusive pathway to participate in authentic research (Bangera and Brownell, 2014) and develop skills and knowledge for future careers in the field. This CURE also exposes students to the public health applications of working with foodborne pathogens as well as provides fundamental genomics training applicable to genomic epidemiology (e.g., variant tracking of SARS-COV-2). Students may, as is also described herein, elect to carry out more advanced investigations on their isolates and their genomes. In this paper, we intend to show not only the methods and tools that might be useful for faculty considering implementing a CURE in pathogen genomics, but also a model for how data derived from the isolates and their genomes can be used to address real-world needs and applications in public health genomic epidemiology and in the comparative genomics of foodborne pathogens.

MATERIALS AND METHODS

Course Background and Implementation

The bulk of this research was implemented in a one-semester upper-division laboratory course at James Madison University. The prerequisite for the course is a general microbiology course, both laboratory and lecture. The course consists of two 2 hour-long lab periods per week, as well as regular lab activities outside of the formal lab periods. The course size during this study ranged from 12 to 24 students per semester and has one or two student teaching assistants who have taken the course. Consistent with Auchincloss et al.'s (2014) framework for CURE

[1]https://enterobase.warwick.ac.uk/

design, the course employed a collaborative, iterative, discovery-based approach intended to meaningfully engage students in authentic scientific research comparable to that of the community of practice.The course is divided up into a wet lab module, Module 1, and a computational module, Module 2. In Module 1 students work in teams to collect samples, from stream sediments and from poultry litter; then they enrich, purify, identify, and characterize *S. enterica* from these samples. In Module 2 the teams assess read quality, assemble, serotype, and annotate their isolates' genomes, identify mobile genetic elements, resistance, and virulence genes, etc.

Either module can be implemented independently or they can be employed sequentially over the course of a semester as is done in this course. Details concerning course implementation, including lesson plans for each module, recommended time lines, assessments, etc. are available in Jurgensen et al. (in press). Complete and detailed wet lab and computational protocols, designed and formatted for use in the course, are freely available on the course Open Science Framework (OSF) page[2]. For Module 1, in addition to stream and manure sources of *Salmonella* as described here, *S. enterica* can be isolated from captive or wild reptiles (Marin et al., 2020), amphibians (Ribas and Poonlaphdecha, 2017) and rodents (Meerburg and Kijlstra, 2007; Swanson et al., 2007). Most of the protocols and methods can be modified for use with *E. coli* as well, which can be readily isolated from many urban and rural surface waters. All the work can be done in a typical college microbiology laboratory, albeit following Biosafety Level 2 protocols (see safety documents on OSF[2]). No specialized equipment beyond that found in a typical teaching microbiology laboratory is required, other than perhaps a thermal cycler. Essentially all the work described was carried out by undergraduates each semester over the span of three iterations of the one-semester course, except the nanopore sequencing, phylogenetic analyses, and the advanced aspects of plasmid identification and annotation – which were done by undergraduates and an M.S. student in the research lab – and the Illumina sequencing, which was done by the Virginia D.C.L.S.

Environmental Sample Collection

Stream sediment was collected from seven sites on four streams near James Madison University in the Shenandoah Valley of Virginia. Water temperature, salinity, and conductivity were collected using a Sonde™ probe (YSI Incorporated, OH, United States). Metadata was recorded using the mobile application Epicollect5[3]. Stream sediment was collected by inverting a sterile 50 mL Falcon® tube and inserting it straight down into the sediment with a gloved hand while avoiding plant matter and gravel. Each tube was filled approximately 3/4 full with sediment and water. Sediment samples were stored at 4°C during transport and in the lab until processing. Poultry litter was acquired from a chicken farm in northern Rockingham County, Virginia, housing approximately 150,000 birds. The farmer was provided with a clean plastic container for filling with litter. Litter was stored at room temperature (20–25°C) until processing.

[2]https://osf.io/gxut3/

[3]https://five.epicollect.net/

Isolation of *Salmonella enterica* From Stream Sediment/Poultry Litter

An outline of the methods used to isolate and identify *S. enterica* in sediment and poultry samples is shown in **Figure 1A**. The procedure was based loosely on the United States FDA Bacteriological Analytical Manual *Salmonella* isolation protocol (Andrews et al., 2014). Both sediment and litter were processed in the same manner. Pre-enrichment began within 24 h of sample collection. Fifty grams of sediment or litter were transferred to sterile 250 mL Erlenmeyer flasks in duplicate. One hundred milliliters of buffered peptone water (10 g peptone, 5 g NaCl, 7 g Na_2HPO_4, 3 g KH_2PO_4 per liter) were added to each flask and mixed by swirling. Pre-enrichments were incubated with shaking at 35°C for 16–22 h. After incubation, 1 mL of supernatant was added to screw-cap tubes containing 10 mL of either Tetrathionate (TT) or Rappaport-Vasilliadis (RV) broth. TT was made in one liter batches (5 g polypeptone, 1 g bile salts, 10 g $CaCO_3$, 30 g $Na_2S_2O_3$ per liter) with additional 20 mL iodine-potassium iodide (5 g KI, 6 g iodine resublimated) added. RV was also made in one liter batches consisting of 100 mL magnesium chloride solution (400 g $MgCl_2$ $6H_2O$ per liter) and 10 mL malachite green oxalate solution (0.4 g malachite green oxalate per liter) to one liter of broth base (5 g tryptone, 8 g NaCl, 1.6 g KH_2PO_4 per liter). RV was autoclaved prior to the addition of pre-enrichment. All enrichments were shaken at 42°C for 5 days.

One hundred microliter aliquots from each enrichment were spread-plated onto Xylose Lysine Tergitol-4 (XLT4) agar (Becton Dickinson, Franklin, NJ, United States) and CHROMagar™ *Salmonella* agar (DRG International Inc., Springfield, NJ). Plates were incubated at 35°C for 16–22 h. Putative *Salmonella* colonies on each medium were identified based on morphology and then streaked onto the complementary agar. If *Salmonella*-like morphology was seen on both media, then colonies were streaked onto tryptic soy agar (TSA) (Becton Dickinson, Franklin, NJ, United States) for purification.

Identification of *S. enterica*

Gram-negative, oxidase-negative, catalase- and KOH-positive isolates were grown in an EnteroPluri™ tube (Becton-Dickinson, Franklin Lakes, NJ, United States). The EnteroPluri™ tube allows for the simultaneous inoculation of multiple media types and the execution of 15 separate biological reactions. Isolates identified as *Salmonella* were then subjected to an endpoint PCR using primers targeting the *Salmonella*-specific gene *invA (Malorny et al., 2003)* for confirmation. Briefly, a small number of cells were acquired by touching an inoculating needle to a colony. The cells were added to 5 μL of ddH_2O in a 0.2 mL PCR tube and lysed in a thermocycler at 95°C for 5 minutes. A master mix was made consisting of 12.5 μL of 2X AmpliTaq Gold® (ThermoFisher Scientific, Waltham, MA, United States) (0.625 U AmpliTaq Gold DNA polymerase, 30 mM Tris/HCl, pH 8.05, 100 mM KCl, 400 μM each dNTP, 5 mM $MgCl_2$), one μL of both *invA* 139 primer (5′-GTGAAATTATCGCCACGTTCGGGCAA-3′) and *invA* 141 primer (5′-TCATCGCACCGTCAAAGGAACC-3′) at

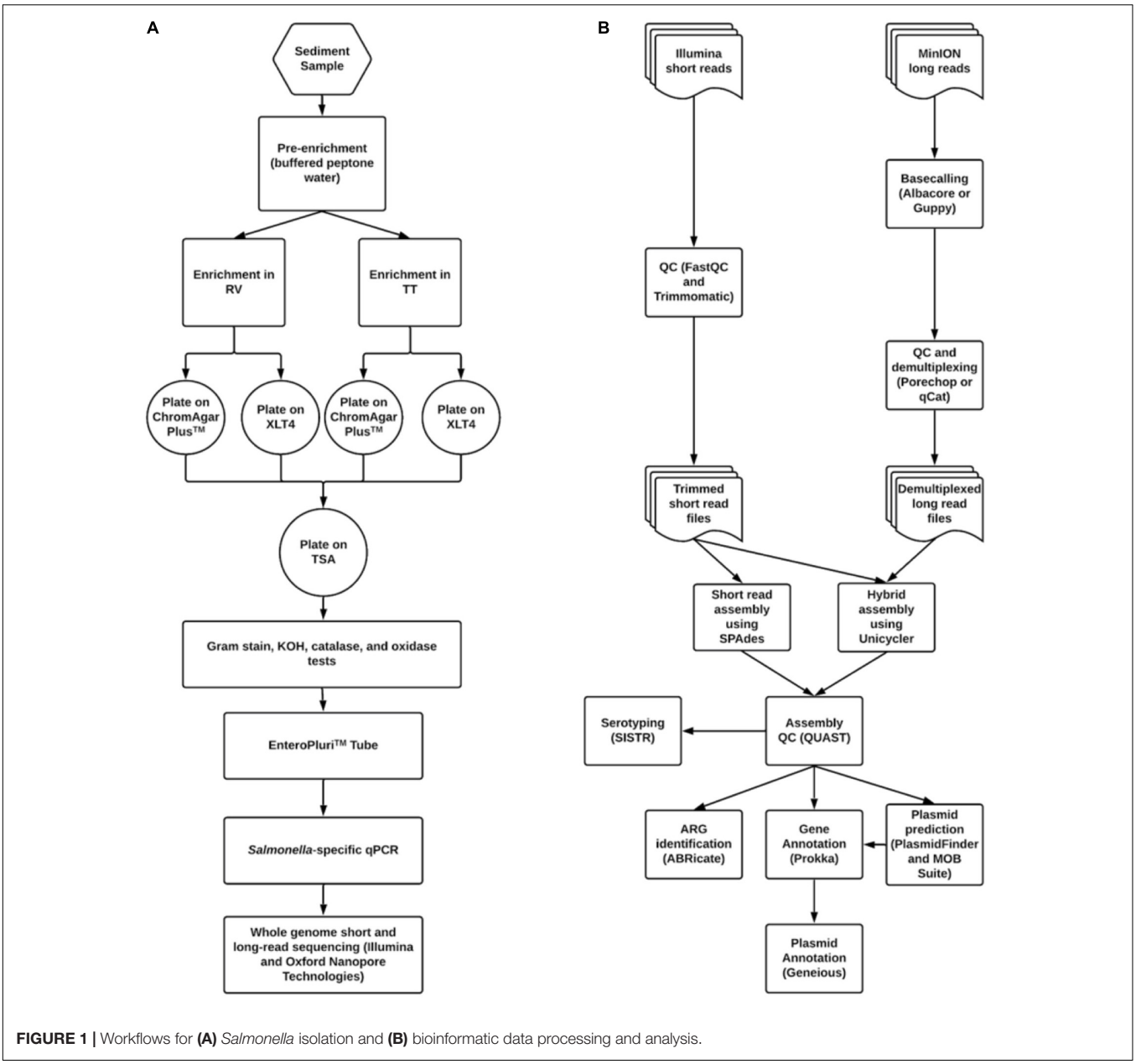

FIGURE 1 | Workflows for **(A)** *Salmonella* isolation and **(B)** bioinformatic data processing and analysis.

10 μM concentrations, and 5 μL of ddH_2O. Twenty microliters of master mix were added to the lysed cell mixture and run according to the program of Malorny et al. (2003): 95°C for one minute followed by 36 cycles of 95°C for 30 s, 64°C for 30 s, and 72°C for 30 s, then a final extension at 72°C for four minutes. For agarose gel electrophoresis, eight microliters of PCR product were added to 2 μL of 5X loading dye prior to loading. The gel was run at 5 V/cm for ca. 120 min and stained with 0.5% GelRed (Biotium Inc., Fremont, CA, United States) for 20-30 min followed by de-staining with ddH_2O for 5 min. Bands were visualized using a UV transilluminator. A band size of 285 bp was expected for an *invA* positive result. For long term storage, one mL of culture was combined with one mL of sterile glycerol in a 2 mL cryogenic freezer tube and stored at −80°C. Strain names were derived from the initials of student teams that isolated them.

Genomic DNA Extraction for Oxford Nanopore DNA Sequencing

Cells were grown in tryptic soy broth for 16–20 h. The Qiagen™ DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD, United States) was used following the manufacturer's instructions for Gram-negative bacteria with some modifications: cell density was not assayed prior to extraction, during the incubation step with proteinase K the length of incubation was kept to a maximum of 1 h, and after elution with ddH_2O, the DNA was left to dissolve for 24 h at 4°C.

Genomic DNA Sequencing

Short read sequence data were generated by the United States FDA Center for Food Safety and Applied Nutrition, as well as the Virginia State Department of Consolidated Laboratory Services. Both carried out sequencing on an Illumina® MiSeq sequencer using either a 300 cycle (2 × 151) or 500 cycle kit (2 × 251). Raw sequence data were uploaded to Illumina BaseSpace, GalaxyTrakr, and to NCBI's Sequence Read Archive (SRA).

For nanopore sequencing, DNA quality and concentrations were assessed using a Synergy H1 Multi-Mode Reader (BioTek Instruments, VT, United States) and a Qubit 2.0 fluorometer, respectively. An $OD_{260/280}$ of 1.8–2.0 was used as the quality cutoff. DNA concentration was determined using the Qubit dsDNA broad range kit. DNA samples were concentrated for samples where when necessary using Microcon® centrifugal filters (Merck Millipore Ltd., MA, United States) according to the manufacturer's instructions.

Prior to nanopore sequencing, a flow cell QC was performed according to the manufacturer's instructions. Library preparation was done using the rapid barcoding kit (SQK-RBK004) according to the manufacturer's instructions (version RNK_9054_v2_revA_23Jan2018; Oxford Nanopore Technologies, Oxford, United Kingdom). Sequencing on the ONT MinION proceeded for up to 48 h using a FLO-MIN106 flowcell (R9.4.1 pore type).

Sequence Data Quality Control and Analysis

Sequence data were processed according to the pipeline shown in **Figure 1B**. Bioinformatic processing and analyses were done in GalaxyTrakr (Gangiredla et al., 2021) or using the command line interface on a computer with an Ubuntu 16.04 LTS operating system. Short read data were quality checked using FastQC[4] version 0.72 or 0.69. Low-quality data was removed using Trimmomatic version 0.36.4 or 0.36.3 (Bolger et al., 2014). Trimmomatic operations consisted of sliding window trimming using a window of four bases with an average quality cutoff of 20, then an overall average quality trimming with a cutoff of 27, and finally a minimum length trimming with a cutoff of 70% the maximum read length (i.e., for data with read length of 251, reads below 75 bp were removed). Trimmed reads were again run through FastQC. Assembly using short read data was carried out using SPAdes version 3.11.1 with default options and specified k-mer values of 21, 33, 55, 77, 99, and 127 (Bankevich et al., 2012).

For long read data, basecalling was performed using either Albacore version 2.2.7 or Guppy 3.0.3 (Oxford Nanopore, Oxford, United Kingdom). Adapter removal and demultiplexing was performed using either Porechop[5] version 0.2.3 or Qcat[6] version 1.0.6. These data were used in conjunction with short read data to carry out a hybrid assembly using Unicycler version 0.4.1.1 (Wick et al., 2017). Assembly quality was assessed using QUAST version 4.6.3 (Gurevich et al., 2013). Assembly quality thresholds used were: N50 > 200,000, number of contigs <200, sequence length ca. 4.4 to 5 Mbp.

Serotyping of *Salmonella* Isolates

Serotyping was done *in silico* using Seqsero2 version 2.0 and SISTR version 1.0.2 on GalaxyTrakr. Additionally, serotyping using SMART PCR (Leader et al., 2009) was carried out on isolates HJ-01 to HJ-26. Trimmed short reads (as FASTQ files) were used as inputs to Seqsero2. Assembled genomes (either short read only or hybrid assemblies, as FASTA files) were used as inputs to SISTR. Agreement between the two *in silico* tools and, if necessary, SMART PCR were used to determine the consensus serotype of a given isolate.

Antimicrobial Resistance Genotyping

Antimicrobial resistance genes (ARGs) were identified using ABRicate[7] versions 0.7.0, 0.8.0, 0.8.7, and ResFinder version 3.2 (Zankari et al., 2012). ABRicate was used through GalaxyTrakr and the command line. Default settings were used on both platforms with two exceptions: a minimum identity cutoff of 80% was specified and the database used was NCBI. ResFinder was run through the Center for Genomic Epidemiology website[8]. For ResFinder, the "acquired ARGs" option was used and final assemblies were submitted.

Phylogenetic Analysis

Eighty-eight *S. enterica* isolates were used to generate a phylogenetic tree in Enterobase (Zhou et al., 2020). A neighbor-joining tree was generated using the algorithm RapidNJ in Enterobase from Enterobase's cgMLST scheme, a set of alleles for 3,002 loci that make up *S. enterica's* core genome. GrapeTree (Zhou et al., 2018) and TreeGraph2 (Stöver and Müller, 2010) were used to visualize the tree. The tree was rooted using the genome of *S. enterica* subspecies *salamae* strain 1315 K. Phandango (Hadfield et al., 2018) was used to visualize source and serotype metadata mapped onto the tree.

In silico Identification and Annotation of Plasmids

MOB_Suite version 1.4.8 (Robertson and Nash, 2018) and PlasmidFinder's most recent version (Carattoli et al., 2014) were used to identify potential plasmids from short read-only assemblies. PlasmidFinder was used through the Center for Genomic Epidemiology website. The Enterobacteriaceae database was employed, with an identity cutoff of 90% and a minimum coverage cutoff of 80%. For MOB_Suite, the mob_recon command's basic options, which require only an input FASTA file and an output directory location, were used, along with the mob_typer command for plasmid typing. MOB_Suite is now available on GalaxyTrakr[9].

Annotation of plasmids was done using the commercial platform Geneious Prime™ 2019 (Biomatters Ltd., San Diego,

[4]https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

[5]https://github.com/rrwick/Porechop

[6]https://github.com/nanoporetech/qcat

[7]https://github.com/tseemann/abricate

[8]http://www.genomicepidemiology.org/

[9]http://www.galaxytrakr.org

CA, United States). Bandage (Wick et al., 2015) was used to visualize assemblies and to identify potential plasmids from hybrid assembly graphs generated by Unicycler. Plasmid sequences were downloaded as FASTA files from Bandage graphs and submitted to Prokka version 0.13.0 (Seemann, 2014) for automatic annotation using the default parameters: Locus tag prefix PROKKA, Locus tag counter increment = 1, GFF version = 3, no forced GenBank/ENA/DDJB compliance, minimum contig size = 200, kingdom = Bacteria, genetic code = 11, similarity e-value cut-off = 0.000001. Not used: "gene" feature for "CDS" feature, genus-specific BLAST database, improve gene prediction for highly fragmented genomes, fast mode, searching for ncRNAs, rRNA search with Barnap, and tRNA search with Aragorn. In addition, identification of unidentified coding sequences was attempted using BLASTx. Parameters used for BLASTx were: max target sequences = 100, expect threshold = 10, word size = 6, and maximum number of matches in a query = 0. The BLOSUM62 matrix was used for scoring. GFF3 files from Prokka were uploaded to Geneious for plasmid mapping and editing.

RESULTS

Course Development

Due to the large amount of poultry and cattle farming in the central Shenandoah Valley of Virginia, we hypothesized that *Salmonella* could be isolated from area streams that drain these farms. If this proved to be true, we intended to develop an elective upper-level research course in which undergraduates would learn to isolate and identify *Salmonella* and to assemble and annotate their genomes. We hoped thereby also to initiate a long-term study on *Salmonella* in streams in our area and to develop the methods necessary for other colleges and universities to replicate the course or a portion thereof. Our focus was particularly on the sediments of streams, as these have been shown to harbor a more stable population of introduced bacteria, particularly *Enterobacteriaceae*, than does the water column itself (Hendricks, 1971; Burton et al., 1987; Pachepsky and Shelton, 2011). Initial attempts to isolate *Salmonella* from sediments in various agriculturally impacted streams were unsuccessful. However, after lengthening the time of enrichment from 24 h to 5 days (**Figure 1A**) we were able to routinely isolate *S. enterica* from all of our tested stream sites.

We wished to use WGS of these isolates both to understand the population-level dynamics of these *Salmonella* and to train students in some basic bioinformatics methods in microbial genomics. A bioinformatics workflow was developed and used for the QC, assembly, and annotation of *Salmonella* genomes (**Figure 1B**). This workflow was carried out primarily using GalaxyTrakr, an FDA Galaxy instance developed particularly for the use of public health laboratories for analyzing the genomes of foodborne pathogens (Gangiredla et al., 2021).

These isolation and computational protocols were piloted in a new course, BIO346 *Bacterial Discovery,* beginning in the spring of 2018. Over three separate semesters, CURE students (n = 52) isolated and characterized 15 *S. enterica* strains. Three of these students went on to isolate an additional 34 strains in the Herrick research lab. These combined with the 39 strains isolated during the methods development for the course resulted in a total of 88 strains isolated from October 2016 through September 2018 (**Table 1**). Of these, 83 strains were isolated from the sediment of seven sites on four streams and five strains from a broiler poultry house, all in the Shenandoah River watershed Rockingham County, Virginia.

Distribution of Sero- and Genotypes

Serotypes were determined by consensus of both SeqSero2 and SISTR and, for strains HJ1 to HJ26, using SMART PCR (Leader et al., 2009). SeqSero2 was unable to serotype strains HJ-02, HJ-04, HJ-13, and HJ-20; however, SMART PCR agreed with SISTR in its serotype determination for these isolates. The 88 isolates were distributed in 19 serotypes, of which the largest number (n = 16) were Typhimurium. The serotypes exactly aligned with the seven-gene multilocus sequence type (MLST), i.e., each serotype corresponded with exactly one MLST. It was found that not only were different serotypes isolated from within the same source, but the same serotype was isolated from different sources as well (**Figure 2**). Also, while certain serotypes were confined to a single source (e.g., Hadar, Bareilly, and Meleagridis), others such as Typhimurium, Montevideo, and Cerro were isolated from a number of different sources.

Among the 88 isolates, 75 distinct core-genome multilocus sequence types (cgMLST) were found (**Table 1**). Thirteen were apparently duplicates, having the same cgMLST as at least one other strain isolated from the same source on the same date. However, six serotype Braenderup strains with the same cgMLST (#4601; strains HJ-30, -32, and-35, PPL-01 and -02, and WEK-03) were isolated from different areas of Cooks Creek over the course of three samplings in 1 year. They were isolated in October 2017 (HJ-30 and HJ-32) and January 2018 (HJ-35) from the upstream CCP site, and in September 2018 the same sequence type was isolated three times (PPL-01, PPL-2, and WEK-03) downstream at the CC11 sampling site (**Table 1**).

Antibiotic Resistance and Plasmid Characterization

Of the 88 isolates collected, 31 were found to contain one or more ARGs (data not shown). Of the 31 isolates with ARGs, 26 were predicted using MOB_Suite and/or PlasmidFinder to house one or more plasmids (data not shown). However, both these tools use assemblies based on short read data as inputs. Short read sequencing data, though highly accurate, result in fragmented, discontiguous assemblies (**Figure 3A**). A hybrid, whole genome assembly approach, incorporating both short and long read data, allowed for the resolving of complete or nearly complete genomes (**Figure 3B**).

Plasmids identified in isolates that contained one or more ARGs were annotated using Prokka (Seemann, 2014). Two of these plasmids are shown in **Figures 4**, **5**. Plasmid pHJ-38

TABLE 1 | *Salmonella enterica* strains isolated from stream sediment and poultry litter in the Shenandoah Valley of Virginia from October 2016 through September 2018.

Isolate	SRR number	Collection date	Source[a]	Source type	Serotype	MLST[b]	cgMLST[c]
HJ-01	SRR5886281	2016/10/02	MC	Sediment	Give	654	97788
HJ-02	SRR5886286	2016/10/02	MC	Sediment	Give	654	80760
HJ-03	SRR5886299	2016/10/02	PR	Sediment	Uganda	684	80845
HJ-04	SRR5886298	2016/10/02	PR	Sediment	Uganda	684	70491
HJ-05	SRR5886283	2016/10/16	CC11	Sediment	Litchfield	214	80915
HJ-06	SRR5886290	2016/10/16	CC11	Sediment	Schwarzengrund	96	80849
HJ-07	SRR5886279	2016/10/16	PR	Sediment	Muenster	321	80796
HJ-08	SRR5886351	2016/10/16	PR	Sediment	Muenster	321	80796
HJ-09	SRR5886350	2016/10/16	CC704	Sediment	Mbandaka	413	80794
HJ-10	SRR5884063	2016/12/05	MC	Sediment	Anatum	64	80537
HJ-11	SRR5884068	2016/12/05	CC704	Sediment	Schwarzengrund	96	80539
HJ-12	SRR5884053	2016/12/05	CC704	Sediment	Senftenberg	14	80536
HJ-13	SRR5884069	2017/01/15	CC11	Sediment	Hadar	33	80533
HJ-14	SRR5884070	2017/01/15	CC11	Sediment	Hadar	33	80527
HJ-15	SRR5884058	2017/01/15	CC11	Sediment	Hadar	33	80538
HJ-16	SRR5884066	2017/02/01	L	Litter	Cerro	367	80542
HJ-17	SRR5884067	2017/02/01	L	Litter	Typhimurium	19	80529
HJ-18	SRR5884056	2017/02/01	L	Litter	Typhimurium	19	80535
HJ-19	SRR5884062	2017/02/01	L	Litter	Typhimurium	19	80530
HJ-20	SRR5884079	2017/02/01	L	Litter	Typhimurium	19	80534
HJ-21	SRR5884080, SRR13268785[d]	2017/02/05	CC11	Sediment	Typhimurium	19	80528
HJ-22	SRR5884077	2017/02/26	CC704	Sediment	Muenchen	112	80532
HJ-23	SRR5884081	2017/02/26	CC704	Sediment	Muenchen	112	80526
HJ-24	SRR6832866, SRR13268782[d]	2017/09/10	PR	Sediment	Montevideo	138	111799
HJ-25	SRR6832877	2017/09/10	PR	Sediment	Montevideo	138	169196
HJ-26	SRR6832873	2017/09/10	MC	Sediment	Senftenberg	14	111800
HJ-27	SRR6366729	2017/10/22	CC704	Sediment	Cerro	367	101373
HJ-28	SRR6367403	2017/10/22	CC704	Sediment	Cerro	367	101373
HJ-29	SRR6369106, SRR13268783[d]	2017/10/22	CC704	Sediment	Anatum	64	11895
HJ-30	SRR6367413	2017/10/22	CCP	Sediment	Braenderup	22	4601
HJ-31	SRR6367404	2017/10/22	CCP	Sediment	Braenderup	22	101420
HJ-32	SRR6367414	2017/10/22	CCP	Sediment	Braenderup	22	4601
HJ-33	SRR6367467	2017/10/22	MC	Sediment	Montevideo	138	101411
HJ-34	SRR6832876	2018/01/21	CCP	Sediment	Braenderup	22	113399
HJ-35	SRR6832896	2018/01/21	CCP	Sediment	Braenderup	22	4601
HJ-36	SRR6832925	2018/01/21	CCP	Sediment	Typhimurium	19	113353
HJ-37	SRR6832911	2018/01/21	CCP	Sediment	Typhimurium	19	111797
HJ-38	SRR6832904, SRR13268784[d]	2018/01/21	CCP	Sediment	Typhimurium	19	111798
HJ-39	SRR6832916	2018/01/21	CCP	Sediment	Typhimurium	19	111391
FHS-01	SRR6832910	2018/01/23	CC704	Sediment	Montevideo	138	166542
FHS-02	SRR6832913, SRR13268786[d]	2018/01/23	CC704	Sediment	Infantis	32	111896
FHS-04	SRR6832912	2018/01/23	CC704	Sediment	Montevideo	138	152383
DG-01	SRR8360264	2018/05/20	PR	Sediment	Muenchen	112	131452
DG-02	SRR8360271	2018/05/20	PR	Sediment	Montevideo	138	131598
DG-03	SRR8104581	2018/05/20	PR	Sediment	Montevideo	138	131599
DG-04	SRR8104579	2018/05/20	CC11	Sediment	Hadar	33	131627
DG-05	SRR8104582	2018/05/20	CC11	Sediment	Hadar	33	131627
DG-06	SRR7504390	2018/05/20	CC11	Sediment	Hadar	33	131627
DG-07	SRR7506701	2018/05/20	CC11	Sediment	Hadar	33	136579
DG-08	SRR7506695	2018/05/20	CC704	Sediment	Typhimurium	19	136587
DG-09	SRR7506699	2018/05/20	CC704	Sediment	Typhimurium	19	131813
DG-10	SRR8360260	2018/05/20	CC704	Sediment	Typhimurium	19	153131
DG-11	SRR7504359	2018/05/20	CC704	Sediment	Typhimurium	19	153155

(Continued)

TABLE 1 | Continued

Isolate	SRR number	Collection date	Source[a]	Source type	Serotype	MLST[b]	cgMLST[c]
DG-12	SRR7506710	2018/05/20	CC704	Sediment	Typhimurium	19	153158
DG-13	SRR7499244	2018/05/20	CC704	Sediment	Typhimurium	19	153152
DG-14	SRR7499253	2018/05/20	MC	Sediment	Meleagridis	463	135814
DG-15	SRR7499245	2018/05/20	MC	Sediment	Meleagridis	463	135813
DG-16	SRR7499272	2018/05/20	MC	Sediment	Cerro	367	135849
DG-17	SRR7499280	2018/05/20	MC	Sediment	Meleagridis	463	135804
DG-18	SRR7499278	2018/05/20	MC	Sediment	Meleagridis	463	135809
DG-19	SRR7889322	2018/05/20	CC11	Sediment	Schwarzengrund	96	144617
DG-20	SRR7878396	2018/05/20	CC704	Sediment	Alachua	1298	144057
DG-21	SRR7889352	2018/05/20	CCP	Sediment	Mbandaka	413	144638
DG-22	SRR7878395	2018/05/20	BR	Sediment	Mbandaka	413	144059
AP-01	SRR8179982	2018/09/03	CC11	Sediment	Bareilly	2553	153210
AP-02	SRR8179943	2018/09/03	CC11	Sediment	Bareilly	2553	153210
AP-03	SRR8179958	2018/09/03	CC11	Sediment	Bareilly	2553	153210
AP-04	SRR8179911	2018/09/03	CC11	Sediment	Bareilly	2553	153210
BES-01	SRR8179966	2018/09/03	CC704	Sediment	Reading	412	153206
BES-02	SRR8179907	2018/09/03	CC11	Sediment	Reading	412	153208
PPL-01	SRR8179980	2018/09/03	CC11	Sediment	Braenderup	22	4601
PPL-02	SRR8179892	2018/09/03	CC11	Sediment	Braenderup	22	4601
WEK-01	SRR8179901	2018/09/03	CC704	Sediment	Montevideo	138	153130
WEK-02	SRR8179936	2018/09/03	CC11	Sediment	Mbandaka	413	153196
WEK-03	SRR8179927	2018/09/03	CC11	Sediment	Braenderup	22	4601
WEK-04	SRR8179981	2018/09/03	CC11	Sediment	Mbandaka	413	153188
WMD-01	SRR8570270	2018/09/30	PR	Sediment	Muenchen	112	164635
WMD-02	SRR8570265	2018/09/30	CC11	Sediment	Give	654	169281
WMD-03	SRR8570271	2018/09/30	MC	Sediment	Montevideo	138	164641
WMD-04	SRR8570267	2018/09/30	MC	Sediment	Montevideo	138	164640
WMD-05	SRR8570264	2018/09/30	CCA	Sediment	Muenster	321	164639
WMD-07	SRR8573695	2018/09/30	PR	Sediment	Montevideo	138	164637
WMD-08	SRR8570269	2018/09/30	CCA	Sediment	Montevideo	138	164636
WMD-09	SRR8570273	2018/09/30	MC	Sediment	Montevideo	138	164643
WMD-10	SRR8570263	2018/09/30	CCA	Sediment	Cerro	367	164638
WMD-11	SRR8570268	2018/09/30	MC	Sediment	Typhimurium	19	164642
WMD-12	SRR8570266	2018/09/30	BR	Sediment	Give	654	164644
WMD-13	SRR8570272	2018/09/30	CCA	Sediment	Cerro	367	164645

[a] *PR: Pleasant Run, CC11: Cook's Creek Rt. 11, CC704: Cook's Creek Rt. 704, CCP: Cook's Creek Park, CCA: Cook's Creek Arboretum, MC: Muddy Creek, BR: Black's Run, and L: poultry litter.*
[b] *MLST: 7-gene multilocus sequence type.*
[c] *cgMLST: core-genome multilocus sequence type, Enterobase scheme (Zhou et al., 2018).*
[d] *Oxford Nanopore fastq SRR numbers.*

was an IncA/C2 plasmid found to have multiple ARGs as predicted by ABRicate and ResFinder (**Figure 4**). Other notable features identified were multiple heavy metal resistance genes and *tra* genes. *Tra* genes are essential for plasmid conjugation between potential host bacteria (Zatyka and Thomas, 1998; Thomas, 2000) and suggest this is a self-transmissible plasmid. Similarly, in the IncF1B plasmid pFHS-02, multiple antibiotic and heavy metal resistance genes were identified (**Figure 5**). Along with *tra* genes, *pil* genes were also present in both plasmids. These genes encode a different pilus than those typically encoded by *tra* genes, one usually associated with conjugation in liquid environments (Bradley, 1984; Zatyka and Thomas, 1998). Plasmid pFHS-02 was also found to contain multiple toxin-antitoxin (or "plasmid addiction") systems. These systems exist to ensure a plasmid's successful replication during host cell division (Hayes, 2003).

Assessment of Educational Outcomes

To determine the impact of the CURE on the development of participants' content knowledge, skills, and attitudes in the domain, direct measures of classroom performance (see Jurgensen et al., in press for an extended discussion of the employed assessments) were partnered with indirect self-report data collected via in-class surveys near the beginning and end of the term. During the first 2 weeks of class, students were asked to complete a short set of closed-response items regarding their academic and demographic background with a limited number of open-ended questions focused on their course expectations.

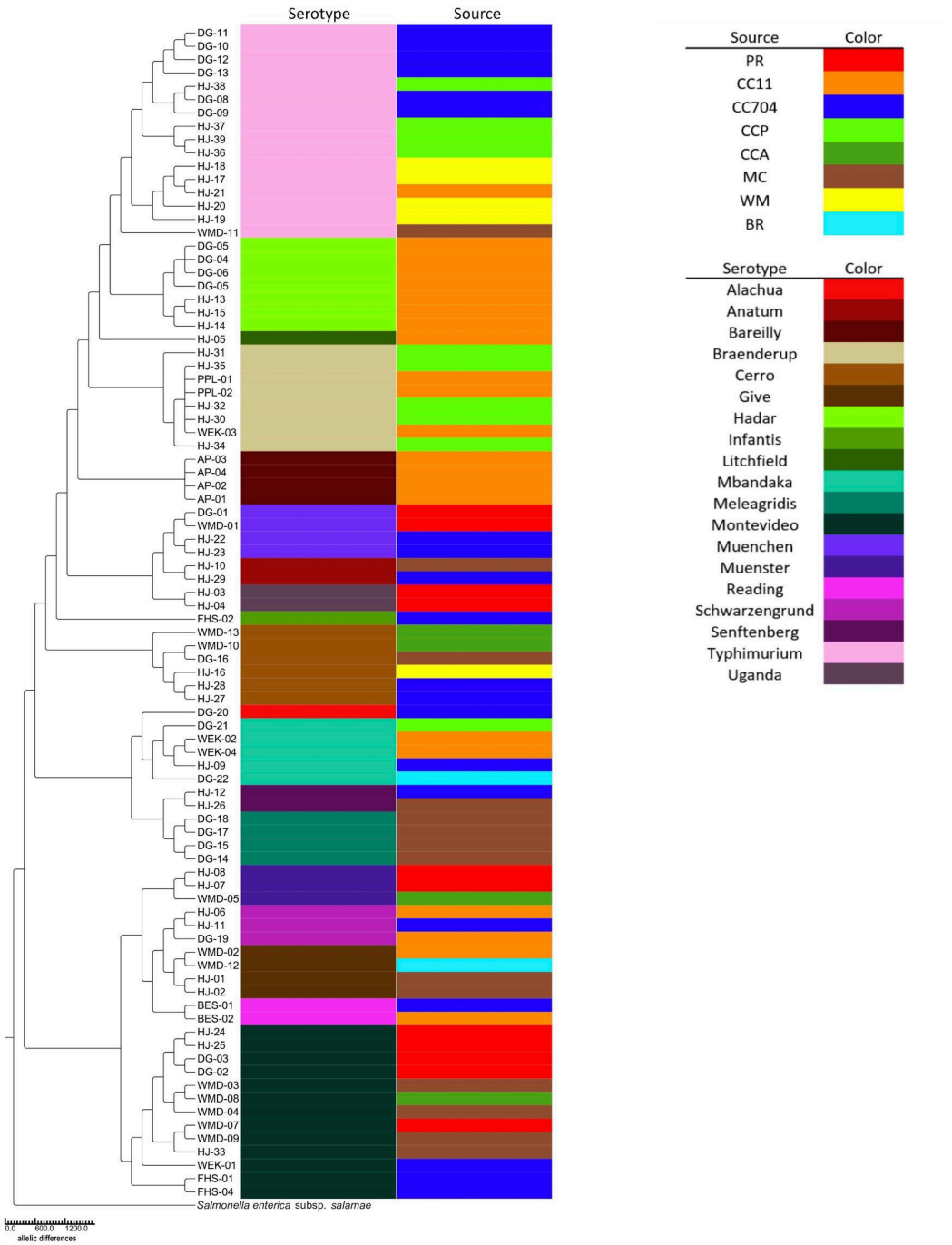

FIGURE 2 | Neighbor-joining cgMLST phylogenetic tree of 88 *S. enterica* isolates generated using the Enterobase GrapeTree RapidNJ algorithm. Branch lengths correspond to the number of allelic differences between isolates. The tree was rooted using *S. enterica* subspecies *salamae* strain 1315K. Isolate source and serotype are indicated by colored bars.

At the end of the term, students responded to Likert scale questions drawn from multiple validated instruments (Hurtado and Carter, 1997; Smith et al., 2013; Corwin et al., 2015b; Hanauer et al., 2017; Maltese et al., 2017) combined with closed-response and open-response questions designed for this study to capture data pertaining to changes in academic/career interests and course design. Survey data were collected online and aggregately analyzed from students in three consecutive semesters of the course (fall 2018, spring 2019, fall 2019) with approval by the university's Institutional Review Board (IRB). Student participants (n = 50) were all biology majors with 74% self-identifying as female, 72% white, 30% first-generation students,

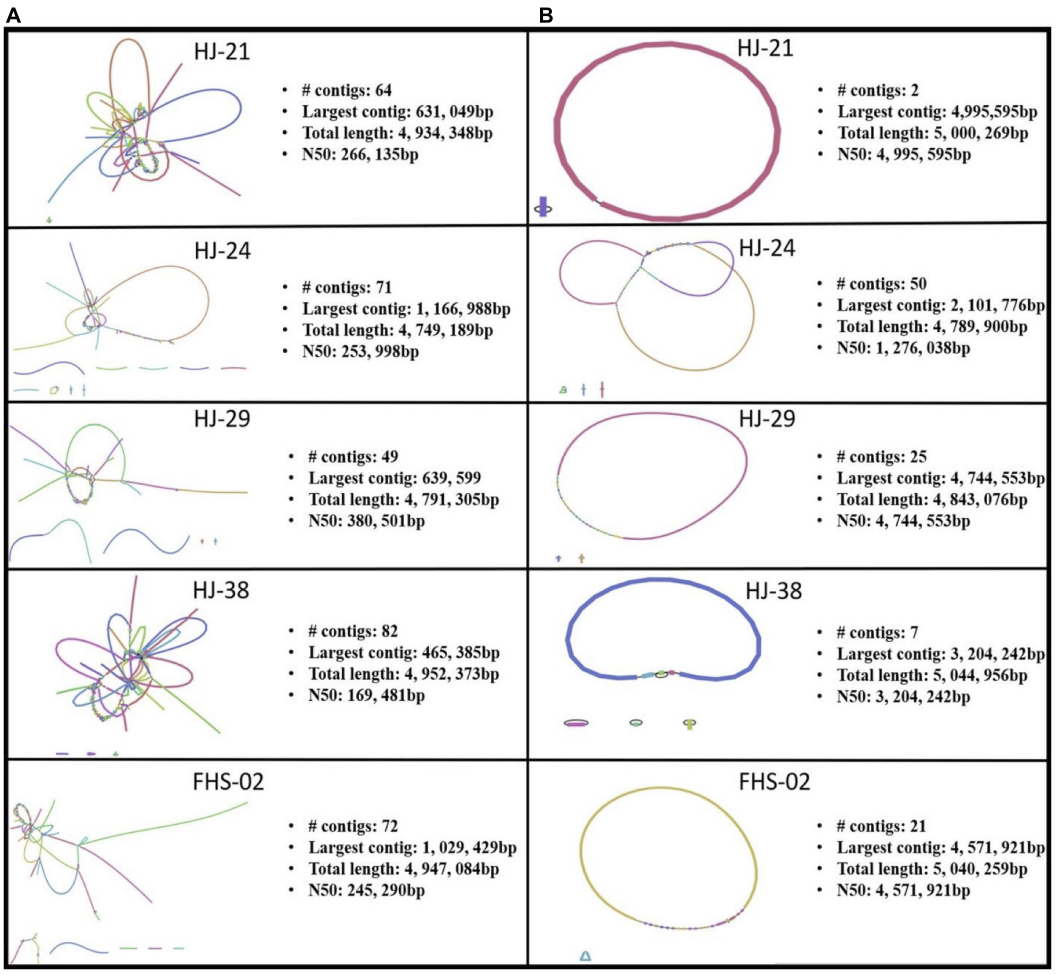

FIGURE 3 | Bandage visualizations of five *Salmonella enterica* plasmid assemblies using **(A)** short read sequence data only, and **(B)** hybrid assemblies using short and long read data.

8% had participated in an apprenticeship-like undergraduate research experience, and 26% were working >15 h per week "to make ends meet," which suggests the course offered an opportunity to engage students who may not be able to volunteer as a traditional, mentored undergraduate researcher due to financial considerations (Bangera and Brownell, 2014). Here, given the focus of this special issue, we report on a subset of post-survey items used to address questions as to how participation in the research course influenced students' academic/career interest and persistence in science, while a description of conferred cognitive outcomes and perceptions of the course is presented in Jurgensen et al. (in press).

Measures

Multiple data points were collected to gain an understanding of how the experience influenced students' academic and career interests. First, using an approach comparable to Maltese and Harsh (2015), students were asked at the onset and end of term to report their intentions (e.g., STEM graduate school, professional school, non-STEM career, and unsure) upon graduation. Exiting students were also prompted if their future intentions had shifted over the term, and if so, they were asked to qualitatively describe whether the course had influenced their plans. Additionally, we also asked students to rate their level of interest in research and science, in general, after the class on a five-point scale. Then, students completed eight parallel items using five-point Likert-type scales (1 = far less interested, 5 = far more interested) created for this project that assessed their interest in pursuing future coursework and research opportunities in specific course topics (i.e., microbiology, molecular biology, bioinformatics, genomics, and microbial ecology) via the stem prompt, "After this class, I am [interest level] in future [coursework or research opportunities] in [topic]." Finally, we used the Persistence in the Sciences survey (PITS) and measures of sense of belonging to assess conferred psychosocial outcomes to research course participants that are relevant to STEM persistence (Chemers et al., 2011; Estrada et al., 2011). The PITS instrument (Hanauer et al., 2016) is designed to measure psychological variables related to student persistence (e.g., project-ownership, self-efficacy, science identity), and has been previously used to assess the effectiveness

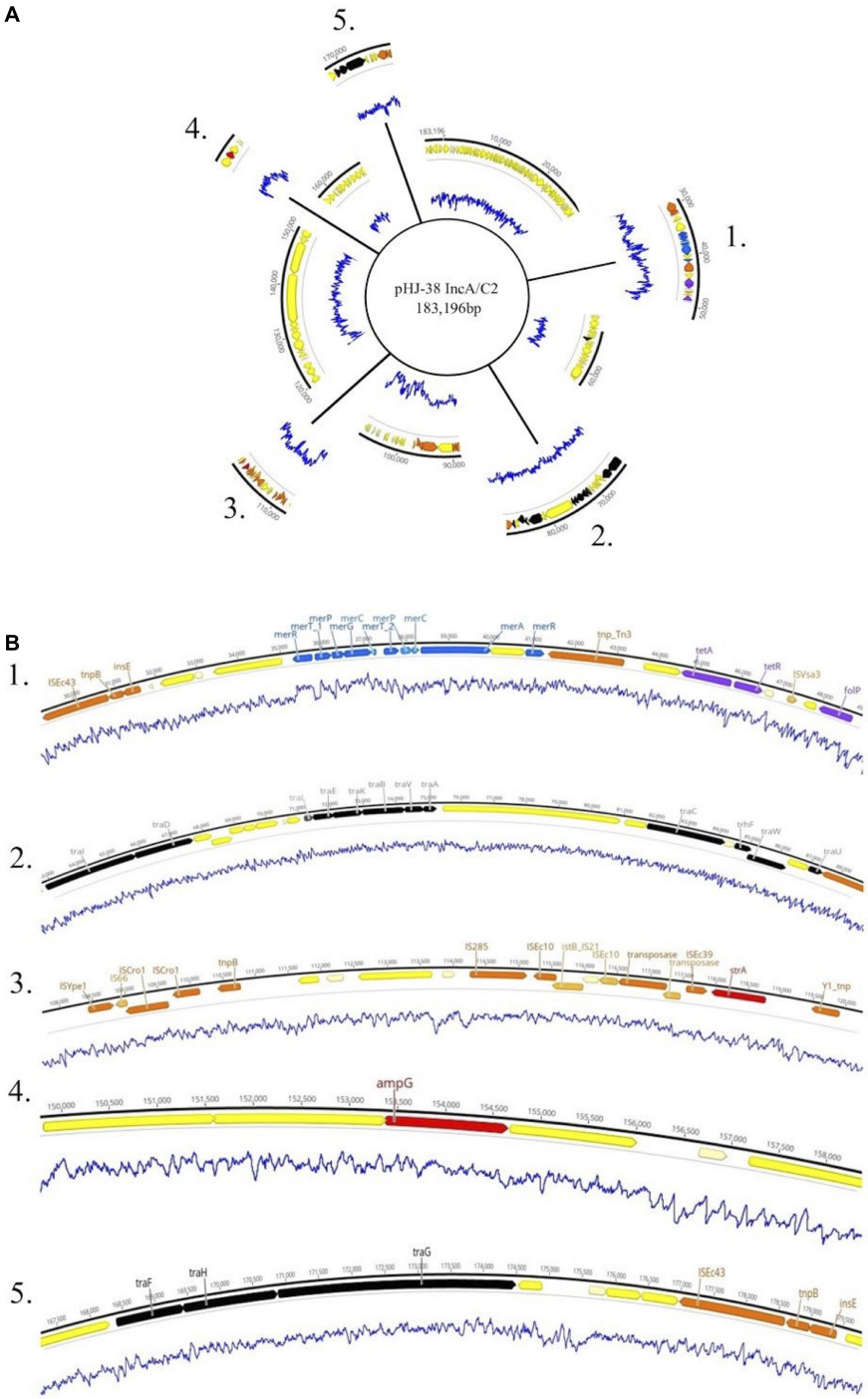

FIGURE 4 | (A) Genetic map of the 184 kb IncA/C2 plasmid pHJ-38. Excised regions 1–5 of the plasmid contain mobility associated genes and genetic elements, antimicrobial and metal resistance genes, virulence genes, and other regions of interest. **(B)** Annotated gene regions containing heavy metal resistance genes (blue); virulence factors (light green); colicin genes (dark green); toxin-antitoxin gene cassettes (pink); genes associated with transposons, IS elements, or integrons (orange); and conjugation genes (black) are shown. Antimicrobial resistance genes identified by both Prokka and Abricate (purple) or by Prokka alone (red) are also shown. Yellow and cream-colored gene regions indicate hypothetical proteins. Blue lines indicate percent GC content.

of CURE educational designs (Hanauer et al., 2017; Corwin et al., 2018; Cooper et al., 2019; Zelaya et al., 2020). Each item is scored on a scale ranging from one to four or five (depending on variable), and then averaged to form a composite variable score. In addition, we also used items modified from Hurtado and Carter (1997) and Smith et al. (2013) to measure

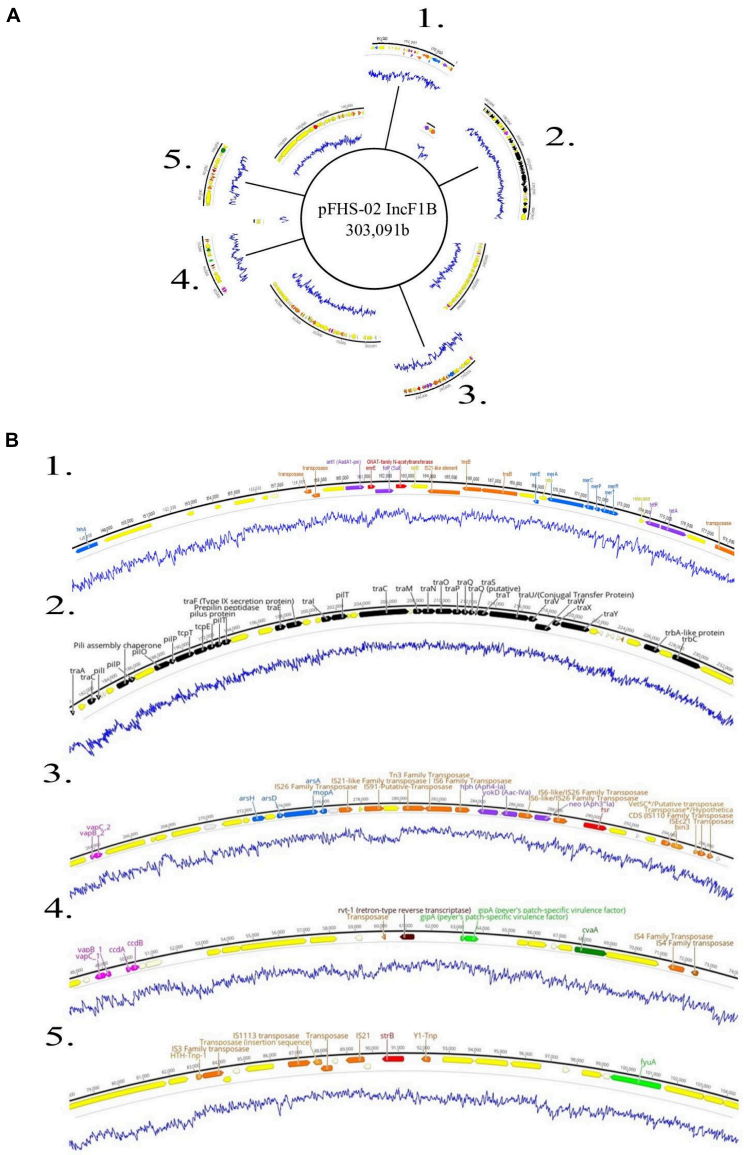

FIGURE 5 | (A) Genetic map of the 303 kb IncF1B plasmid pFHS-02. Regions 1–5 of the plasmid containing mobility associated genes and genetic elements, antimicrobial and metal resistance genes, virulence genes, and other regions of interest are shown excised. **(B)** Annotated gene regions; genes and labels are colored as indicated in **Figure 4**. Blue lines indicate percent GC content.

sense of belonging assessed using five-point Likert-type scales (1 = strongly disagree, 5 = highly agree).

Findings

Students entered the course largely with the intentions of attending graduate school in a STEM field (42%) or professional school (42%) after degree completion, with fewer intending to seek immediate employment in STEM fields (12%) or who were unsure of their respective plans (4%). Fifty-six percent of participants reported their academic or career plans changed to some degree during the term, with 36% of all students specifically identifying that the course influenced their future intentions. Most often, similar to interest shifts observed in mentored undergraduate research experience (Maltese and Harsh, 2015), students indicated that the experience refined or triggered their interest through exposure to fields of study and careers related to genomic epidemiology. For instance, two students stated in open-ended questions that "*[The course] opened the door and exposed me to genomics and bioinformatics in which I had never thought about in my future career*" and "*It significantly influenced my future plans and [sic] considering to attend a graduate school specifically [to study] foodborne pathogens in relation to the environment so that I possibly could work for the CDC or FDA in the future.*" A notable proportion of students upon exiting the course indicated that the experience enhanced their interest, to some degree, in

coursework in bioinformatics (40%), microbial ecology (33%), molecular biology (24%), and genomics (22%). While the balance of respondents largely reported no change in their prior interests, a small subset (<10%) indicated decreased interests in these topical areas resultant of class participation. A modest shift in interest in microbiology coursework was also noted (8% increase, 92% no change), which likely reflects the preexisting interests of students that opted into such an upper-level course. Results also showed that 77% percent of students identified that course participation increased their overall interest in pursuing future research opportunities. More specifically, a fair proportion of students indicated that the experience enhanced their interests, to some degree, in research in bioinformatics (42% of respondents), molecular biology (34%), genomics (31%), and microbial ecology (24%) research with a smaller shift for microbiology (16%). On the other hand, approximately 20% of students indicated that they became less interested in research over the term in the respective areas of microbial ecology, bioinformatics, and genomics. Together, these results suggest that the research course refined student interest by the opportunity to test the proverbial waters of different fields through authentic practice, which may guide their later academic and career intentions in a means comparable to that of a traditional undergraduate research experience (Hunter et al., 2007; Maltese and Harsh, 2015).

In addition to observing shifts in academic and/or career interests, we sought to assess the impact of course participation on psychosocial aspects often correlated with academic success and STEM persistence (Perez et al., 2014; Trujillo and Tanner, 2014). Most students (80, 72, and 76%, respectively) reported on the post-survey that participation in the course directly contributed to a greater sense of belonging to the department, campus community, and scientific community. Students reported high ratings on PITS items measuring their science-identity (M = 4.52, SD = 0.46), self-efficacy (M = 4.32, SD = 0.77), cognitive project ownership (M = 4.22, SD = 0.59), and networking (M = 4.10, SD = 0.71) as related to course participation. These ratings are comparable to or higher than those reported in previously published studies on biologically focused CUREs (Corwin et al., 2015a; Hanauer et al., 2017; Cooper et al., 2019; Zelaya et al., 2020) that use the same instruments (Hanauer and Dolan, 2014; Hanauer et al., 2016) to assess student outcomes. The overall pattern of early findings suggests the research course contributes to psychosocial outcomes that influence STEM persistence, though additional data is needed to allow comparisons to be drawn between groups to assess the impact of participation of the experience on *all* students.

DISCUSSION

Animal husbandry plays a prominent role in the economy of the Shenandoah Valley of Virginia, with an estimated 159 million chickens, 16 million turkeys, and over a half million cattle raised in 2012 in four counties alone. These were estimated to produce over 400 tons and 1.28 billion gallons of manure in that year[10]. We hypothesized that agricultural runoff, particularly from poultry and cattle, would result in detectable enteric pathogens such as *Salmonella* in these streams and rivers. In the approximately 15 months before beginning this course, and then over three semesters of the course itself, the sediments of seven sites on four streams and one poultry house were sampled and a total of 88 distinct *S. enterica* strains were isolated. Standard microbiological techniques along with the Enteropluri tube and PCR of the *invA* gene were used to verify the isolates' identity (**Figure 1A**). Students typically characterized their isolates further by examining phenotypic antibiotic resistance (using Kirby-Bauer or Sensititre^{TM} MIC panels) and by isolating native plasmids (Heringa et al., 2007). After short read Illumina sequencing, students in the course used the FDA's GalaxyTrakr web platform for sequence trimming and filtering, assembly of draft genomes, sequence and genome assembly quality control, annotation of assemblies, and *in silico* serotyping. GalaxyTrakr is an instance of Galaxy[11] that was developed by the FDA as a bioinformatics platform for use by United States public health laboratories. However, it is particularly advantageous for educational use, as the computational tools included are only those typically used for studying microbial genomes in general, particularly those of pathogens (Gangiredla et al., 2021). After they assembled, annotated, and serotyped their isolate genomes, students were then asked to pursue specific questions and hypotheses related to genes and gene functional categories of interest in their isolates, including those relating to antibiotic resistance, virulence, phages, plasmids, transposons, etc.

The 88 strains of *S. enterica* represented 19 unique serotypes. In the CDC's report on the top 10 serotypes responsible for human infections in the United States[12], five of the ten – Typhimurium, Infantis, Muenchen, Montevideo, and Braenderup – were repeatedly isolated in this study. Interestingly, a notable serotype that was not isolated was Enteritidis. This was surprising because Enteritidis is one of the most common serotypes associated with poultry[13], yet no samples near or even directly from a poultry farm yielded this serotype.

Enterobase was used to identify 75 distinct core genome multilocus sequence types (cgMLSTs) among the 88 isolates (**Table 1** and **Figure 2**). The cgMLST implemented in Enterobase is a highly discriminatory typing scheme. It reflects a so-called "soft-core genome" which in *Salmonella* consists of 3,002 genes found in ≥98%, intact in ≥94% and of "unexceptional diversity" in over 3,000 *Salmonella* genomes (Alikhan et al., 2018). A single base-pair difference in any of these genes would result in a different cgMLST. Six Braenderup isolates of cgMLST 4601 were isolated at different times and from different portions of Cooks Creek, with three isolates found upstream and three isolated

[10]https://environmentalintegrity.org/reports/water-pollution-from-livestock-in-the-shenandoah-valley/

[11]http://www.usegalaxy.org

[12]https://www.cdc.gov/nationalsurveillance/pdfs/2016-Salmonella-report-508.pdf

[13]https://www.fsis.usda.gov/wps/portal/fsis/topics/data-collection-and-reports/microbiology/annual-serotyping-reports

9–11 months later from an area ca. 7.5 km downstream. Since all belonged to the same core genome sequence type, there was no detected divergence between the isolates (**Figure 2**), suggesting that they may have come from a common source. It is possible that members of the same population of *Salmonella* from the upstream site moved down to the downstream site, or that there were independent introductions of the same type, perhaps from poultry litter spread on fields within the watershed.

Five strains were further sequenced using the Oxford Nanopore MinION^{TM} and hybrid-assembled to yield complete or near-complete circular assemblies. Two of these plasmids, pHJ38 and pFHS-02, were annotated and found to have multiple ARGs and two types of pilus genes potentially facilitating conjugation under multiple environmental conditions (**Figures 3**, **4**). Plasmid pFHS-02 was particularly notable as it was a very large (303 kb) megaplasmid containing 11 predicted ARGs, 11 predicted heavy metal (mercury, arsenic, tellurite, and molybdenum) resistance genes, four predicted toxin-antitoxin systems, multiple transposons and IS elements, and a phage-associated virulence gene (*gipA*) associated with Peyer's patch colonization and macrophage survival (Stanley et al., 2000; Vazeille et al., 2016). We have previously isolated numerous large, self-transmissible multidrug-resistant plasmids from many of these streams (Herrick et al., 2014).

We present here a model laboratory course design for introducing upper level microbiology undergraduates to real-world public health and pathogen surveillance methods and applications, as well as to laboratory research techniques and computational biology methods. At our university, this elective one-semester course is offered to students who have taken a course in general microbiology, including laboratory. Most of these students are majors in biology, typically with a concentration in microbiology, although some are allied health students majoring in health science, nursing, etc.

The course is divided into separate modules – one on the wet lab methods used for *Salmonella* isolation, isolation, and identification, and the other covering bioinformatics techniques. Linking the two modules is the *gratis* sequencing provided, in our case, by the United States FDA and our state public health laboratory, the Virginia DCLS. Foodborne and other related pathogens are of interest to many regional, state, and national public health laboratories for genomic epidemiological surveillance and these agencies are often willing, even eager, to sequence these at no cost. Although Module 1, focusing on the isolation of *Salmonella*, requires knowledge and skills in general microbiology laboratory techniques, the computational methods of Module 2 could conceivably be utilized by students with only a background in general college biology concepts. Module 2 in particular can be employed as a standalone research experience. Students could download the raw reads of *Salmonella* or other pathogens that are readily available from the NCBI Sequence Read Archive and work with them using the Bioinformatics Lab Guides available for this course[2].

Salmonella enterica is one of the leading causes of foodborne illness in the world (Majowicz et al., 2010). It is also one of the most-sequenced organisms on earth[2], due primarily to the massive efforts of agencies like the United States Food and Drug Administration and individual United States state public health laboratories. These and other national and regional agencies are interested in tracking the occurrence and spread of *S. enterica* and other pathogenic bacteria (Allard et al., 2018) and therefore are often willing to sequence, at no cost, the whole genomes of isolated strains, especially those isolated from less-sampled sources. However, public health agencies' interest in the huge number of *Salmonella*, *E. coli,* Clostridioides, *Vibrio*, and other pathogens being sequenced worldwide rarely extends beyond cataloging their genomes for possible future epidemiological use. There is therefore a vast repository of essentially unanalyzed raw sequencing reads that have never been analyzed beyond a simple automated assembly and annotation, let alone examined for important accessory and other genetic elements such as plasmids, phages, transposons, ARGs, CRISPR regions, or virulence genes. This opens up an opportunity for students to work on authentic and important problems not only in genomic epidemiology and pathogen surveillance but also in mobile gene transfer, antibiotic resistance, the evolution of virulence, microbial ecology, etc. Students can potentially use and analyze either (1) strains they isolate themselves, or (2) the archived pathogen genomes in NCBI and other genome repositories. Depending on their needs, instructors could deploy a course such as this as a whole or as separate modules, one focusing on the wet lab isolation, identification, and characterization of *Salmonella* and the other on the genomics of *Salmonella* or other pathogens. Although *Salmonella* is a Biosafety II level (BSL 2) pathogen, it is relatively safe to work with in a classroom setting. It is not uncommonly cultivated for use in upper-division and even general microbiology laboratory courses (Ponder and Sumner, 2009; Marvasi et al., 2015). We have recently published details on safely setting up and using either module or the course in its entirety (Jurgensen et al., in press). We have also established an OSF page containing protocols, bioinformatics guides, safety documents, posters, etc. related to the course (see text footnote 1).

Over three-quarters of the students who took the course indicated they developed increased interest in research. Over 40% were interested in pursuing further study or research in bioinformatics and genomics and more than a third said that it influenced their future academic or career plans, particularly in relation to pathogen genomics and genomic epidemiology. Students in the course also benefited from their interaction with our research lab, where we are seeking to understand more specifically the ecology of the *Enterobacteriaceae* in secondary habitats such as streams, as well as their evolution via horizontal gene transfer. This course serves as a "feeder" of both data and interested students to more advanced research projects. Graduate and advanced undergraduate students from our research lab also serve as mentors to students in the course, especially with the more advanced aspects of their projects. Data generated from the course has been presented in a regional symposium[14] and used in thesis projects (Jurgensen, 2018; Greenman, 2019).

This course provided a unique opportunity for microbiology students to gain valuable skills in pathogen isolation and

[14]http://www.asmbranches.org/brva/uploads/2/1/5/8/21583328/2018_asmva_final_program.pdf

identification, and in the basics of WGS and microbial genomics. Students were also introduced to the applications of these methods in public health microbiology, genomic epidemiology, pathogen surveillance, and in genome research in general. Course-based research experiences such as this can provide many of the benefits of traditional mentor-guided, open-ended and authentic research to students who might not otherwise have such an opportunity. They can also provide opportunities for students who have the interest to pursue further and deeper research questions on their isolates and their genomes, questions that are directly applicable to genomic epidemiology and to understanding the genomics and ecology of foodborne pathogens in the environment.

DATA AVAILABILITY STATEMENT

Illumina raw sequencing reads were deposited in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA186035 for isolates HJ-01 through HJ-23 and BioProject PRJNA219491 for all others. Nanopore sequences for isolates HJ-21, HJ-24, HJ-29, HJ-38, and FHS-02 have been deposited in the SRA under BioProject PRJNA605356. SRR identifiers can be found in **Table 1**.

ETHICS STATEMENT

Procedures involved in the collection and analysis of student data to assess the efficacy and impact of the research course, including the informed consent process and confidentiality parameters, were reviewed and approved by the James Madison University's Institutional Review Board (IRB No. 18-0508).

AUTHOR CONTRIBUTIONS

JBH conceived the project. NAG, SKJ, CPH, CJK, and JBH designed the experiments. SKJ, CPH, CJK, RED, WMM, and JBH developed the methods. SKJ, RED, WMM, and JBH developed the instructional aspects of the *Bacterial Discovery* course. NAG, SKJ, CPH, CJK, RED, WMM, DE, BAE, SMH, TNK, HGL, DJL, and BW performed the experiments and conducted bioinformatics data analysis (undergraduates DE, BAE, TNK, HGL, DJL, and BW carried these out during the course itself). JAH gathered, compiled, and analyzed student assessment data. NAG, JBH, and JAH wrote the manuscript. SKJ, CJK, and RED edited it. The figures and table were generated by NAG. JSK, CPH, RED, WMM, DE, BAE, SMH, TNK, HGL, DJL, and BW were undergraduates at the time this work was done, NAG and CJK were graduate students, and JAH and JBH were on the faculty at James Madison University. All authors have read and approved the final manuscript.

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REFERENCES

Alikhan, N.-F., Zhou, Z., Sergeant, M. J., and Achtman, M. (2018). A genomic overview of the population structure of *Salmonella*. *PLoS Genet.* 14:e1007261. doi: 10.1371/journal.pgen.1007261

Allard, M. W., Bell, R., Ferreira, C. M., Gonzalez-Escalona, N., Hoffmann, M., Muruvanda, T., et al. (2018). Genomics of foodborne pathogens for microbial food safety. *Curr. Opin. Biotechnol.* 49, 224–229.

American Association for the Advancement of Science (2011). *Vision and Change in Undergraduate Biology Education: a Call to Action*. Washington, DC: American Association for the Advancement of Science.

Andrews, W. H., Jacobson, A., and Hammack, T. (2014). *Bacteriological Analytical Manual: Salmonella*. Silver Spring: U.S. Food and Drug Administration.

Armstrong, G. L., MacCannell, D. R., Taylor, J., Carleton, H. A., Neuhaus, E. B., Bradbury, R. S., et al. (2019). Pathogen genomics in public health. *N. Engl. J. Med.* 381, 2569–2580.

Auchincloss, L. C., Laursen, S. L., Branchaw, J. L., Eagan, K., Graham, M., Hanauer, D. I., et al. (2014). Assessment of course-based undergraduate research experiences: a meeting report. *CBE Life Sci. Educ.* 13, 29–40. doi: 10.1187/cbe.14-01-0004

Bangera, G., and Brownell, S. E. (2014). Course-based undergraduate research experiences can make scientific research more inclusive. *CBE Life Sci. Educ.* 13, 602–606. doi: 10.1187/cbe.14-06-0099

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., et al. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19, 455–477. doi: 10.1089/cmb.2012.0021

Bell, R. L., Zheng, J., Burrows, E., Allard, S., Wang, C. Y., Keys, C. E., et al. (2015). Ecological prevalence, genetic diversity, and epidemiological aspects of *Salmonella* isolated from tomato agricultural regions of the Virginia Eastern Shore. *Front. Microbiol.* 6:415. doi: 10.3389/fmicb.2015.00415

Bhunia, A. K. (2018). "*Salmonella enterica*," in *Foodborne Microbial Pathogens: Mechanisms and Pathogenesis*, ed. A. K. Bhunia (New York, NY: Springer), 271–287.

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. doi: 10.1093/bioinformatics/btu170

Bradley, D. E. (1984). Characteristics and function of thick and thin conjugative pili determined by transfer-derepressed plasmids of incompatibility groups I1.

I2, I5, B, K and Z. J. *Gen. Microbiol.* 130, 1489–1502. doi: 10.1099/00221287-130-6-1489

Burton, G. A. Jr., Gunnison, D., and Lanza, G. R. (1987). Survival of pathogenic bacteria in various freshwater sediments. *Appl. Environ. Microbiol.* 53, 633–638. doi: 10.1128/aem.53.4.633-638.1987

Carattoli, A., Zankari, E., Garciá-Fernández, A., Larsen, M. V., Lund, O., Villa, L., et al. (2014). *In silico* detection and typing of plasmids using Plasmidfinder and plasmid multilocus sequence typing. *Antimicrob. Agents Chemother.* 58, 3895–3903. doi: 10.1128/aac.02412-14

Chemers, M. M., Zurbriggen, E. L., Syed, M., Goza, B. K., and Bearman, S. (2011). The role of efficacy and identity in science career commitment among underrepresented minority students: efficacy and identity in science career commitment. *J. Soc. Issues* 67, 469–491. doi: 10.1111/j.1540-4560.2011.01710.x

Cooper, K. M., Blattman, J. N., Hendrix, T., and Brownell, S. E. (2019). The impact of broadly relevant novel discoveries on student project ownership in a traditional lab course turned cure. *CBE Life Sci. Educ.* 18:ar57. doi: 10.1187/cbe.19-06-0113

Corwin, L. A., Graham, M. J., and Dolan, E. L. (2015a). Modeling course-based undergraduate research experiences: an agenda for future research and evaluation. *CBE Life Sci. Educ.* 14:es1. doi: 10.1187/cbe.14-10-0167

Corwin, L. A., Runyon, C., Robinson, A., and Dolan, E. L. (2015b). The laboratory course assessment survey: a tool to measure three dimensions of research-course design. *CBE Life Sci. Educ.* 14:ar37. doi: 10.1187/cbe.15-03-0073

Corwin, L. A., Runyon, C. R., Ghanem, E., Sandy, M., Clark, G., Palmer, G. C., et al. (2018). Effects of discovery, iteration, and collaboration in laboratory courses on undergraduates' research career intentions fully mediated by student ownership. *CBE Life Sci. Educ.* 17:ar20. doi: 10.1187/cbe.17-07-0141

Crump, J. A., Sjölund-Karlsson, M., Gordon, M. A., and Parry, C. M. (2015). Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clin. Microbiol. Rev.* 28, 901–937. doi: 10.1128/cmr.00002-15

Deng, X., den Bakker, H. C., and Hendriksen, R. S. (2016). Genomic epidemiology: whole-genome-sequencing–powered surveillance and outbreak investigation of foodborne bacterial pathogens. *Annu. Rev. Food Sci. Technol.* 7, 353–374. doi: 10.1146/annurev-food-041715-033259

Estrada, M., Woodcock, A., Hernandez, P. R., and Schultz, P. W. (2011). Toward a model of social influence that explains minority student integration into the scientific community. *J. Educ. Psychol.* 103, 206–222. doi: 10.1037/a0020743

Gangiredla, J., Rand, H., Benisatto, D., Payne, J., Strittmatter, C., Sanders, J., et al. (2021). GalaxyTrakr: a distributed analysis tool for public health whole genome sequence data accessible to non-bioinformaticians. *BMC Genomics* 22:114. doi: 10.1186/s12864-021-07405-8

Greenman, N. (2019). Assessing the prevalence of multidrug resistant Salmonella enterica from stream sediment and poultry litter in the Shenandoah Valley of Virginia. *Masters Theses, 2010–2019*. 594. Available online at: https://commons.lib.jmu.edu/master201019/594.

Gurevich, A., Saveliev, V., Vyahhi, N., and Tesler, G. (2013). QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29, 1072–1075. doi: 10.1093/bioinformatics/btt086

Hadfield, J., Croucher, N. J., Goater, R. J., Abudahab, K., Aanensen, D. M., and Harris, S. R. (2018). Phandango: an interactive viewer for bacterial population genomics. *Bioinformatics* 34, 292–293. doi: 10.1093/bioinformatics/btx610

Hanauer, D. I., and Dolan, E. L. (2014). The project ownership survey: measuring differences in scientific inquiry experiences. *CBE—Life Sci. Educ.* 13, 149–158. doi: 10.1187/cbe.13-06-0123

Hanauer, D. I., Graham, M. J., and Hatfull, G. F. (2016). A measure of college student persistence in the sciences (PITS). *CBE Life Sci. Educ.* 15:ar54. doi: 10.1187/cbe.15-09-0185

Hanauer, D. I., Graham, M. J., Sea-Phages, Betancur, L., Bobrownicki, A., Cresawn, S. G., et al. (2017). An inclusive Research Education Community (iREC): impact of the SEA-PHAGES program on research outcomes and student learning. *Proc. Natl. Acad. Sci. U S A.* 114, 13531–13536. doi: 10.1073/pnas.1718188115

Hayes, F. (2003). Toxins-antitoxins: plasmid maintenance, programmed cell death, and cell cycle arrest. *Science* 301, 1496–1499. doi: 10.1126/science.1088157

Hendricks, C. W. (1971). Increased recovery rate of *Salmonellae* from stream bottom sediments versus surface waters. *Appl. Microbiol.* 21, 379–380. doi: 10.1128/am.21.2.379-380.1971

Heringa, S. D., Monroe, J. D., and Herrick, J. B. (2007). A simple, rapid method for extracting large plasmid DNA from bacteria. *Nat Prec*. doi: 10.1038/npre.2007.1249.1

Herrick, J. B., Haynes, R., Heringa, S., Brooks, J. M., and Sobota, L. T. (2014). Coselection for resistance to multiple late-generation human therapeutic antibiotics encoded on tetracycline resistance plasmids captured from uncultivated stream and soil bacteria. *J. Appl. Microbiol.* 117, 380–389. doi: 10.1111/jam.12538

Hunter, A.-B., Laursen, S. L., and Seymour, E. (2007). Becoming a scientist: the role of undergraduate research in students' cognitive, personal, and professional development. *Sci. Educ.* 91, 36–74. doi: 10.1002/sce.20173

Hurtado, S., and Carter, D. F. (1997). Effects of college transition and perceptions of the campus racial climate on latino college students'. *Sense Belonging. Sociol. Educ.* 70, 324–345. doi: 10.2307/2673270

Jurgensen, S. (2018). A cure for salmonella: engaging students in pathogen microbiology and bioinformatics. *Senior Honors Projects, 2010–2019*. 623. Available online at: https://commons.lib.jmu.edu/honors201019/623

Jurgensen, S. K., Harsh, J., and Herrick, J. B. (in press). *). A CURE for Salmonella: A Laboratory Course in Pathogen Microbiology and Genomics*. *CourseSource*.

Leader, B. T., Frye, J. G., Hu, J., Fedorka-Cray, P. J., and Boyle, D. S. (2009). High-throughput molecular determination of *salmonella enterica* serovars by use of multiplex PCR and capillary electrophoresis analysis. *J. Clin. Microbiol.* 47, 1290–1299. doi: 10.1128/jcm.02095-08

Levantesi, C., Bonadonna, L., Briancesco, R., Grohmann, E., Toze, S., and Tandoi, V. (2012). *Salmonella* in surface and drinking water: occurrence and water-mediated transmission. *Food Res. Int.* 45, 587–602. doi: 10.1016/j.foodres.2011.06.037

Liu, H., Whitehouse, C. A., and Li, B. (2018). Presence and persistence of *Salmonella* in water: the impact on microbial quality of water and food safety. *Front Public Health* 6:159. doi: 10.3389/fpubh.2018.00159

Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'Brien, S. J., et al. (2010). The global burden of nontyphoidal *Salmonella gastroenteritis*. *Clin. Infect. Dis.* 50, 882–889.

Malorny, B., Hoorfar, J., Bunge, C., and Helmuth, R. (2003). Multicenter validation of the analytical accuracy of *Salmonella* PCR: towards an international standard. *Appl. Environ. Microbiol.* 69, 290–296. doi: 10.1128/aem.69.1.290-296.2003

Maltese, A. V., and Harsh, J. A. (2015). *Pathways of Entry into STEM Across K–16. Interest and the Self in K-16 Mathematics and Science Learning*, Washington, DC: American Educational Research Association.

Maltese, A., Harsh, J., and Jung, E. (2017). Evaluating undergraduate research experiences—development of a self-report tool. *Educ. Sci.* 7:87. doi: 10.3390/educsci7040087

Marin, C., Lorenzo-Rebenaque, L., Laso, O., Villora-Gonzalez, J., and Vega, S. (2020). Pet reptiles: a potential source of transmission of multidrug-resistant *Salmonella*. *Front. Vet. Sci.* 7:613718. doi: 10.3389/fvets.2020.613718

Marvasi, M., Choudhury, M., and Teplitski, M. (2015). Laboratory activity to teach about the proliferation of *Salmonella* in vegetables. *J. Microbiol. Biol. Educ.* 16, 230–236. doi: 10.1128/jmbe.v16i2.948

Meerburg, B. G., and Kijlstra, A. (2007). Role of rodents in transmission of *Salmonella* and *Campylobacter*. *J. Sci. Food Agric.* 87, 2774–2781.

Nadon, C., Van Walle, I., Gerner-Smidt, P., Campos, J., Chinen, I., Concepcion-Acevedo, J., et al. (2017). PulseNet international: vision for the implementation of whole genome sequencing (WGS) for global food-borne disease surveillance. *Euro. Surveill.* 22:30544. doi: 10.2807/1560-7917.ES.2017.22.23.30544

National Academies of Sciences, Engineering, and Medicine (2017). *Undergraduate Research Experiences for STEM Students: Successes, Challenges, and Opportunities*. Washington, D.C: National Academies Press.

National Research Council (US) Committee on Undergraduate Biology Education to Prepare Research Scientists for the 21st Century (2003). *BIO2010: Transforming Undergraduate Education for Future Research Biologists*. Washington, D.C: National Academies Press.

Pachepsky, Y. A., and Shelton, D. R. (2011). *Escherichia coli* and fecal coliforms in freshwater and estuarine sediments. *Crit. Rev. Environ. Sci. Technol.* 41, 1067–1110. doi: 10.1080/10643380903392718

Parisi, A., Stanaway, J. D., Sarkar, K., and Crump, J. A. (2020). The global burden of non-typhoidal *Salmonella* invasive disease: a systematic analysis for the Global Burden of Disease Study 2017. *Int. J. Infect. Dis.* 101:341. doi: 10.1016/j.ijid.2020.09.898

Perez, T., Cromley, J. G., and Kaplan, A. (2014). The role of identity development, values, and costs in college STEM retention. *J. Educ. Psychol.* 106, 315–329. doi: 10.1037/a0034027

Ponder, M. A., and Sumner, S. (2009). Use of case studies to introduce undergraduate students to principles of food microbiology, molecular biology, and epidemiology of food-borne disease. *Biochem. Mol. Biol. Educ.* 37, 156–163. doi: 10.1002/bmb.20277

Ribas, A., and Poonlaphdecha, S. (2017). Wild-caught and farm-reared amphibians are important reservoirs of *Salmonella*, a study in North-East Thailand. *Zoonoses Public Health* 64, 106–110. doi: 10.1111/zph.12286

Robertson, J., and Nash, J. H. E. (2018). MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. *Microbial Genom.* 4:e000206. doi: 10.1099/mgen.0.000206

Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30, 2068–2069. doi: 10.1093/bioinformatics/btu153

Silva, C., Maloy, S., and Calva, E. (2014). One health and food-borne disease: *Salmonella* transmission between humans, animals, and plants. *Microbiol. Spectrum* 2, 137–148. doi: 10.1128/microbiolspec.oh-0020-2013

Smith, J. L., Lewis, K. L., Hawthorne, L., and Hodges, S. D. (2013). When trying hard isn't natural: women's belonging with and motivation for male-dominated STEM fields as a function of effort expenditure concerns. *Pers. Soc. Psychol. Bull.* 39, 131–143. doi: 10.1177/0146167212468332

Stanley, T. L., Ellermeier, C. D., and Slauch, J. M. (2000). Tissue-specific gene expression identifies a gene in the lysogenic phage Gifsy-1 that affects *Salmonella enterica* serovar typhimurium survival in Peyer's patches. *J. Bacteriol.* 182, 4406–4413. doi: 10.1128/jb.182.16.4406-4413.2000

Stöver, B. C., and Müller, K. F. (2010). TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinform.* 11:7. doi: 10.1186/1471-2105-11-7

Swanson, S. J., Snider, C., Braden, C. R., Boxrud, D., Wünschmann, A., Rudroff, J. A., et al. (2007). Multidrug-resistant *Salmonella enterica* serotype typhimurium associated with pet rodents. *N. Engl. J. Med.* 356, 21–28.

Tang, P., Croxen, M. A., Hasan, M. R., Hsiao, W. W. L., and Hoang, L. M. (2017). Infection control in the new age of genomic epidemiology. *Am. J. Infect. Control.* 45, 170–179. doi: 10.1016/j.ajic.2016.05.015

Thomas, C. M. (2000). Paradigms of plasmid organization. *Mol. Microbiol.* 37, 485–491. doi: 10.1046/j.1365-2958.2000.02006.x

Trujillo, G., and Tanner, K. D. (2014). Considering the role of affect in learning: monitoring students' self-efficacy, sense of belonging, and science identity. *CBE Life Sci. Educ.* 13, 6–15. doi: 10.1187/cbe.13-12-0241

Vazeille, E., Chassaing, B., Buisson, A., Dubois, A., de Vallée, A., Billard, E., et al. (2016). GipA factor supports colonization of Peyer's patches by Crohn's disease-associated *Escherichia coli*. *Inflamm. Bowel Dis.* 22, 68–81. doi: 10.1097/mib.0000000000000609

Wick, R. R., Judd, L. M., Gorrie, C. L., and Holt, K. E. (2017). Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput. Biol.* 13:e1005595. doi: 10.1371/journal.pcbi.1005595

Wick, R. R., Schultz, M. B., Zobel, J., and Holt, K. E. (2015). Bandage: interactive visualization of de novo genome assemblies. *Bioinformatics* 31, 3350–3352. doi: 10.1093/bioinformatics/btv383

Winfield, M. D., and Groisman, E. A. (2003). Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Appl. Environ. Microbiol.* 69, 3687–3694. doi: 10.1128/aem.69.7.3687-3694.2003

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., et al. (2012). Identification of acquired antimicrobial resistance genes. *J. Antimicrob. Chemother.* 67, 2640–2644. doi: 10.1093/jac/dks261

Zatyka, M., and Thomas, C. M. (1998). Control of genes for conjugative transfer of plasmids and other mobile elements. *FEMS Microbiol. Rev.* 21, 291–319. doi: 10.1111/j.1574-6976.1998.tb00355.x

Zelaya, A. J., Gerardo, N. M., Blumer, L. S., and Beck, C. W. (2020). The bean beetle microbiome project: a course-based undergraduate research experience in microbiology. *Front. Microbiol.* 11:577621. doi: 10.3389/fmicb.2020.577621

Zhou, Z., Alikhan, N.-F., Mohamed, K., Fan, Y., Agama Study, Group, A. S., and Achatman, M. (2020). The EnteroBase user's guide, with case studies on *Salmonella* transmissions, *Yersinia pestis* phylogeny, and *Escherichia* core genomic diversity. *Genome Res.* 30, 138–152. doi: 10.1101/gr.251678.119

Zhou, Z., Alikhan, N.-F., Sergeant, M. J., Luhmann, N., Vaz, C., Francisco, A. P., et al. (2018). GrapeTree: visualization of core genomic relationships among 100,000 bacterial pathogens. *Genome Res.* 28, 1395–1404. doi: 10.1101/gr.232397.117

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Implementation of a Service-Learning Project Focused on Handwashing and Vaccinations Within an Undergraduate Microbiology Laboratory Course

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Two relevant topics in keeping populations healthy are handwashing and vaccinations. Thus, the service-learning project titled "We Are Healthy" campaign was introduced within a microbiology laboratory course with two objectives; our biologists would better understand the importance of these actions by designing activities that engage the student community and to obtain an understanding of the campus community's behaviors and beliefs concerning these topics. Students designed the campaign to include handwashing stations, pictures of bacterial cultures from swabbing common surfaces, and trivia questions testing their peers' knowledge of various vaccines, as well as handwashing and vaccination surveys. To assess the impact of the campaign on microbiology students (n = 34), they were provided 10 questions that were scored on a scale from 1 to 5 (1 = strongly disagree; 5 = strongly agree). Student gains (score > 3) were reported for depth in knowledge, development of better public speaking skills, and greater respect for volunteers suggesting that the campaign was beneficial. This study subsequently led to the receiving of grants that allowed the continuation of the campaign within the course, the securing of funding for handwashing and hand sanitizing stations and the initiation of new undergraduate research projects.

Keywords: service-learning, handwashing, vaccinations, microbiology education, undergraduate

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INTRODUCTION

It is likely that the words "experience" and "leadership" are mentioned multiple times in a typical advising appointment that is focused on career goals. Twenty-five years ago, most lectures consisted of a one-sided, continuous dialog by the instructor and laboratories were designed to provide specific results through a cookie-cutter-type design; thus, students were most likely instructed to gain experience and leadership through extracurricular formats *via* undergraduate research opportunities, jobs, and organizations/clubs. The idea of including these opportunities within the classroom was at the heart of the American Association for the Advancement of Science's (2011) report. By embedding real research projects within classroom laboratories, all students were inevitably pushed into the driver seat of their education through the exploration of novel research questions. Active engagement within these CUREs works to build strong science foundations by focusing on students gaining a deeper understanding of the scientific process as well as

analytical/technical skills (Thiry et al., 2011; Auchincloss et al., 2014). However, to provide students with the best resume for success, undergraduate experiences should not be confined by classroom/laboratory walls and should inspire biologists to become leaders not only within their field but also within their community (Hart, 2015; Webb, 2016; McGowin and Teed, 2019). This is especially important in current times where most would not disagree that there is a disconnection between scientific research and the media. Explanations for the disconnection involve a general underestimation of how difficult it is to connect with diverse audiences, perceptions that the public does not want to understand science, a lack of recognition for outreach/community engagement, and the lack of formal training in undergraduate and graduate curricula (Brownell et al., 2013; Devonshire and Hathway, 2014; Varner, 2014). Following the success of CUREs, it seems that including community engagement within the curricula could provide a new dignity within the field of biology. Service-learning projects aim to fill a need on both sides; a community need is satisfied, and the students gain a wider perspective of their course content and build a connection with their community (Bringle and Hatcher, 1996). This paper briefly describes the incorporation of a service-learning project into a microbiology laboratory course in which students developed a campaign to increase awareness toward two important factors regarding human health, handwashing, and vaccinations.

The idea for a campaign that focused on handwashing was born from many students saying they wash their hands more often after an early laboratory introduced them to the frequency of bacteria around them, the chemistry of handwashing, and a review of proper handwashing techniques. The recent coronavirus pandemic hopefully reminded us all that our hands are important transmission factors in the spread of both respiratory and gastrointestinal infections; thus, the best way to limit pathogen access to our bodies is through good hand hygiene (Drankiewicz and Dundes, 2003; Allegranzi and Pittet, 2009; Mackert et al., 2013). While handwashing is considered as a social norm in developed countries, studies suggest that proper hand hygiene is not occurring (Anderson et al., 2008). In a direct observational study of several public restrooms, only 66.9% washed their hands with soap and only 5% washed for more than 15 s as designated by the CDC (Borchgrevink et al., 2013). Vaccinations were included within this project primarily because the university was changing their policy regarding vaccinations, requiring the measles, mumps, and rubella vaccine for all degree-seeking students and the meningococcal vaccine for all students living on campus; however, this is a major medical issue as anti-vaccine campaigns continue to increase. Vaccines were once proclaimed lifesavers by parents, but the number of vaccine-hesitant parents is growing at an alarming rate. It is estimated that 15% of children are under-immunized with parents doubting the effectiveness of vaccines and having concerns regarding the amount of vaccines children are given before the age of two (Rabinowitz et al., 2016). Additionally, the decrease in immunization rates is a serious concern because it threatens the herd immunity model (Berezin and Eads, 2016; Sobo, 2016). Vaccines can only be effective if a threshold of the population is vaccinated to reduce the transmission rates of the disease lowering the incidence of the disease (Orenstein and Ahmed, 2017). As more parents are choosing to reduce immunization rates, thresholds needed within the population to maintain safety are in jeopardy.

The service-learning event was designated the "We Are Healthy" campaign playing off the popular "We Are…Penn State" phrase that is known by all current and former Penn State students. The campaign was introduced within a microbiology laboratory course with two objectives; our biologists would better understand the importance of these actions by designing activities that engage the student community and obtain an understanding of the campus community's behaviors and beliefs concerning these topics.

MATERIALS AND METHODS

The campaign was incorporated into three sections of the MICRB202 Introduction to Microbiology laboratory course at Penn State Erie in the fall 2017 semester. In the weeks preceding the event, students were asked to do several assignments and discuss the campaign during a portion of the laboratory session. Students were asked to design handwashing advertisements stressing that the good handwashing requires 20 s of scrubbing with soap. The advertisements were then placed throughout the campus (student union eatery and bathrooms). Central to the campaign were handwashing demonstrations. For this, several portable handwashing stations were rented so proper handwashing techniques could be demonstrated using Glitterbug potion and viewing stations (Brevis Corporation). For the vaccine portion of the campaign, groups of 2–4 students were assigned one of four vaccines: MMR, meningitis, human papilloma virus, or influenza, to research and present to the class. From their research, students were asked to develop multiple-choice or true/false questions that could be used as trivia questions during the campaign. Survey questions were developed to understand participants' views regarding handwashing and vaccinations. Handwashing surveys asked when students washed their hands after five daily activities, how long they wash their hands, four agree/disagree statements, and two questions regarding hand sanitizers for a total of 13 questions. Vaccine surveys contained five agree/disagree statements and 11 yes/no questions that focused on some general thoughts toward vaccination programs, an understanding of the term herd immunity, and some specific questions regarding the flu, measles, meningitis, and HPV vaccines (these were chosen because they are either required or highly recommended before attending college). Surveys were done on paper, and participants needed to circle their answers.

Once the main activities were determined, a strategy was needed to get passing-by students to participate in the campaign. Students wanted to use a food that required participants to use their hands rather than a utensil and decided on popcorn. It was also decided that a freebie item, a syringe pen, would

be awarded to those who successfully answered a trivia question. In this strategy, a student assigned as the Go-Getter, would boldly walk up to students, ask them to fill out the surveys, and upon completion, receive a bag of popcorn. Popcorn machines were borrowed from the athletics department and student government association. Another student assigned as the survey collector would instruct students to drop their surveys into a covered box and instruct the participant that they could get their popcorn after they washed their hands. Students assigned as the handwashing police would supply Glitterbug potion and keep the time of handwashing. After properly washing their hands, students were given their popcorn from the student assigned as the popcorn distributor. The student assigned as the trivia host would then ask the student a question for their chance to win a syringe pen. Questions were presented to students in large bold print on a standard piece of paper within a page protector in which the host could read the question and the answer choices were displayed to the participant. For the campaign, microbiology students (6–10) were stationed at two high-traffic areas within our campus and were wearing T-shirts with the campaign logo. Each student had a designated role and participated in the campaign for 2 h, while the event occurred at both sites simultaneously for 4 h.

Results and Discussion

Overall, this event was easily incorporated into an introductory laboratory and additional costs were minimal. The largest costs were purchasing coordinating T-shirts and rental of handwashing stations. To reduce these costs, the campus bathrooms could be used though the timing of 20 s could be harder to maintain. Coordinating shirts are not necessary for such a campaign, but it is helpful and could be arranged by wearing school colors or spirit shirts the students already own. The most common comment from students was that it was harder than they thought to engage with their fellow students. Popcorn and syringe pens were great incentives for engagement, and based on the amount of surveys collected, we interacted with over 400 students. The most common comment from participants was their surprise at how long 20 s is and their admission that they do not typically wash their hands for that long. Participants were also asked to respond to two separate surveys each containing more than ten questions. Due to the number of questions, the results were not completed by the end of the class so shorter surveys would be suggested. It was revealed that less than 50% of the students reported that they washed their hands for the recommended 15–20 s. Students were not surprised by this but seeing the statistics from a group familiar to them made the statistic more "real," and we discussed how they might approach the issue in their future careers. In regard to the vaccination survey, it was shown that most of the participants believe that immunizations are an important part of a healthy lifestyle, but students were quick to point out that the size of the minority that was either neutral or disagreed (11%) with the statement was not ideal. Since participation time is limited in the described campaign, a deeper understanding of vaccination programs by participants would be more likely if the campaign was coordinated with a keynote speaker from the state or county health department.

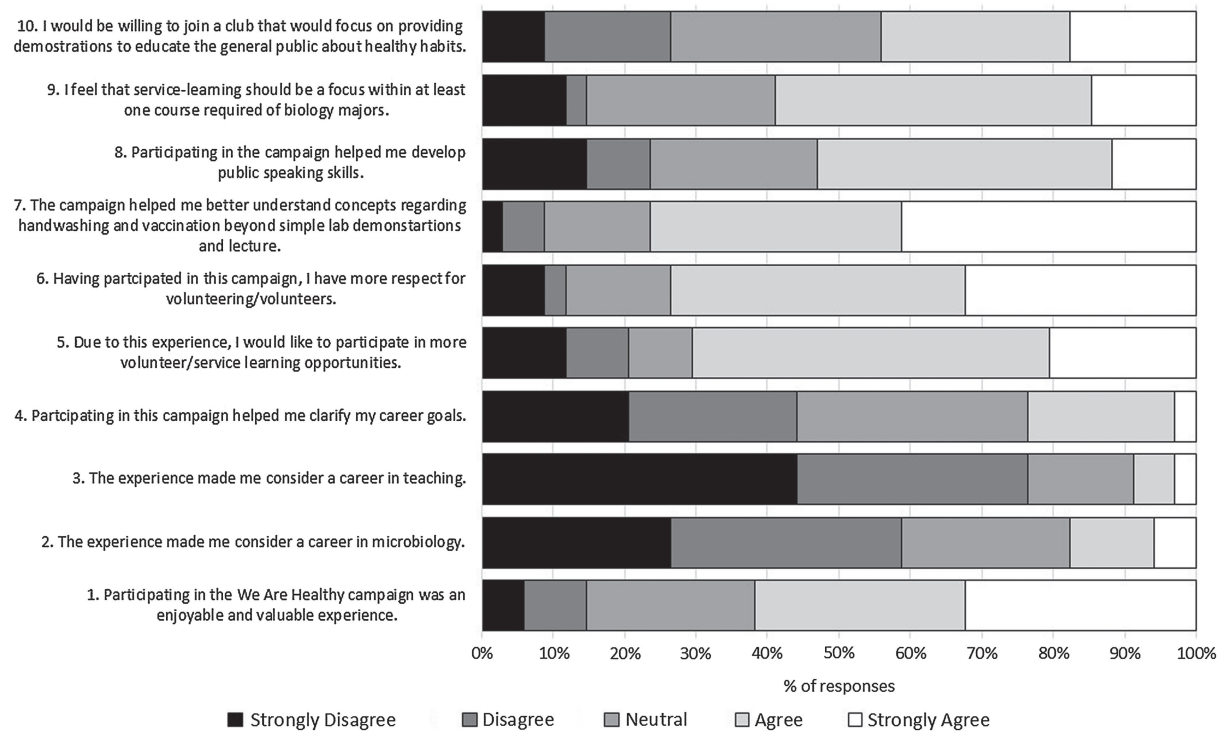

FIGURE 1 | Assessment questions and responses from microbiology students (n = 34) after participation in the "We Are Healthy" campaign.

To assess the impact of the campaign on microbiology students (n = 34), they were provided ten questions which were adapted from questions asked in a published study assessing the inclusion of a service-learning project within a microbiology course (Webb, 2016). Questions were scored on a scale from 1 to 5 (1 = strongly disagree; 5 = strongly agree). **Figure 1** shows student gains (score > 3) were reported for depth in knowledge, development of better public speaking skills, and greater respect for volunteers suggesting that the campaign was beneficial. Little effect was shown on student career choices which may not be surprising as this course is typically taken in their third or fourth year when they have started focusing on specific career goals. Thus, it might be helpful to include service-learning projects earlier within the curriculum, perhaps even during a program-specific first-year seminar experience.

Given the importance of handwashing and vaccinations, this service-learning project could be repeated each year. To add flexibility and allow students more control in the planning, venues could be rotated to include elementary classrooms, after-school programs, and senior care facilities. Since a significant amount of data was collected in the campaign, a compilation of the data was undertaken by several students as part of an undergraduate research experience and presented at a local Sigma Xi conference. The results of this campaign have been used to apply for a few internal grants that have allowed for the continuation of the campaign, purchase of our handwashing station to use in outreach events, an increase in the number of public hand sanitizer stations, and inspired a few students to further understand hygiene behaviors specifically within the campus workout facility.

In conclusion, embedding this service-learning project got students involved in sharing course knowledge with their immediate community in a way that was active and fun for the community *via* engaging demonstrations, trivia questions, and small incentives. The inclusion of course-based service-learning projects as well as research experiences would place experience and leadership opportunities at the core of our curricula and would provide a solid foundation for students to advance the field of biology.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, and further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Penn State Office for Research Protections. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BP conceived and designed the service-learning project and wrote the manuscript.

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REFERENCES

Allegranzi, B., and Pittet, D. (2009). Role of hand hygiene in healthcare-associated infection prevention. *J. Hosp. Infect.* 73, 305–315. doi: 10.1016/j.jhin.2009.04.019

American Association for the Advancement of Science (2011). Vision and change in undergraduate biology education: a call to action, final report. Washington, DC.

Anderson, J. A., Warren, C. A., Perez, E., Louis, R. I., Phillips, S., Wheeler, J., et al. (2008). Gender and ethnic differences in hand hygiene practices among college students. *Am. J. Infect. Control.* 36, 361–368. doi: 10.1016/j.ajic.2007.09.007

Auchincloss, C., Laursen, S. L., Bran chaw, J. L., Eagan, K., Graham, M., Hanauer, D. I., et al. (2014). Assessment of course-based undergraduate research experiences: a meeting report. *CBE—Life Sci. Educ.* 13, 29–40. doi: 10.1187/cbe.14-01-0004

Berezin, M., and Eads, A. (2016). Risk is for the rich? Childhood vaccination resistance and culture of health. *Soc. Sci. Med.* 165, 233–245. doi: 10.1016/j.socscimed.2016.07.009

Borchgrevink, C. P., Cha, J., and Kim, S. (2013). Hand washing practices in a college town environment. *J. Environ. Health* 75, 18–24.

Bringle, R. G., and Hatcher, J. A. (1996). Implementing service learning in higher education. *J. High. Educ.* 67, 221–239. doi: 10.1080/00221546.1996.11780257

Brownell, S. E., Price, J. V., and Steinman, L. (2013). Science communication to the general public: why we need to teach undergraduate and graduate students this skill as part of their formal scientific training. *J. Undergrad. Neurosci. Educ.* 12, 6–10.

Devonshire, I. M., and Hathway, G. J. (2014). Overcoming the barriers to greater public engagement. *PLoS Biol.* 12, 1–4. doi: 10.1371/journal.pbio.1001761

Drankiewicz, B. A., and Dundes, L. (2003). Handwashing among female college students. *Am. J. Infect. Control* 31, 67–71. doi: 10.1067/mic.2003.6

Hart, S. (2015). Engaging the learner: the ABC's of service-learning. *Teach. Learn. Nurs.* 10, 76–79. doi: 10.1016/j.teln.2015.01.001

Mackert, M., Liang, M., and Champlin, S. (2013). Think the sink: preliminary evaluation of a handwashing promotion campaign. *Am. J. Infect. Control* 41, 275–277. doi: 10.1016/j.ajic.2012.03.023

McGowin, A. E., and Teed, R. (2019). Increasing expression of civic-engagement values by students in a service-learning chemistry course. *J. Chem. Educ.* 96, 2158–2166. doi: 10.1021/acs.jchemed.9b00221

Orenstein, W. A., and Ahmed, R. (2017). Simply put: vaccination saves lives. *Proc. Natl. Acad. Sci. U. S. A.* 114, 4031–4033. doi: 10.1073/pnas.1704507114

Rabinowitz, M., Latella, L., Stern, C., Jost, J. T. (2016). Beliefs about childhood vaccination in the united states: political Ideology, false consensus, and the illusion of uniqueness. *PLoS One* 11:e0158382. doi: 10.1371/journal.pone.0158382

Sobo, E. (2016). What is herd immunity and how does it relate to pediatric vaccination uptake? US parent perspectives. *Soc. Sci. Med.* 165, 187–195. doi: 10.1016/j.socscimed.2016.06.015

Thiry, H., Laursen, S. L., and Hunter, A.-B. (2011). What experiences help students become scientists? A comparative study of research and other sources of personal and professional gains for STEM undergraduates. *J. High. Educ.* 82, 357–388. doi: 10.1080/00221546.2011.11777209

Varner, J. (2014). Scientific outreach: toward effective public engagement with biological sciences. *Bioscience* 64, 333–340. doi: 10.1093/biosci/biu021

Webb, G. (2016). Learning through teaching: a microbiology service-learning experience. *J. Microbiol. Biol. Educ.* 17, 86–89. doi: 10.1128/jmbe.v17i1.997

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Service-Learning, Movies, and Infectious Diseases: Implementation of an Active Educational Program in Microbiology as a Tool for Engagement in Social Justice

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Service-Learning is an educational methodology that allows student learning while addressing community needs. A program in microbiology and infectious diseases was implemented in Universidad Complutense de Madrid, Spain. University lecturers, clinical microbiologists, doctorate students, and undergraduates from several Bachelor Degrees and courses worked in an interdisciplinary team along with social institutions that attend disadvantaged persons. Using commercial movies that deal with infectious diseases, the students learn clinical microbiology, prepare divulgation materials, visit social centers to accompany, and help others to know about illnesses and prevention. The program was developed through two academic years and involved 58 voluntary students, 13 teachers and tutors, and 4 social entities as community partners. Postsurvey evaluation of the program revealed a highly satisfactory achievement of goals: acquiring scientific and personal competencies by university students, including critical analysis and science diffusion, solving problems or collaborative team working, and contributing, together with the tutors, to the social responsibility of the university.

Keywords: social justice, infectious diseases, microbiology, service-learning, educational methodology

INTRODUCTION

Disadvantaged or underserved persons have the same infectious diseases as the general population, although they are disproportionately affected by higher rates of acute and chronic illnesses (Grief and Miller, 2017). Several studies in different developed countries recorded an almost 10-fold higher incidence of HIV infection, tuberculosis, and other respiratory infections, or Hepatitis B and C in homeless people when compared to general populations (Sivic et al., 2013; Tornero et al., 2016;

Grief and Miller, 2017; Rudge et al., 2018; Tsai and Wilson, 2020). Other common diseases are skin infections, such as scabies, pediculosis, or tinea, and foot disorders like impetigo or cellulitis (Raoult et al., 2001; Sivic et al., 2013). A lack of hygiene, person-to-person and bedding contact, injuries, and additional problems such as drug or alcohol addiction and mental disorders are some of the reasons for this situation. Similarly, infections and sexually transmitted diseases (STD) affect other groups at risk of social exclusion, i.e., sex workers, intravenous drug addicts, immigrants, or imprisoned (Tornero et al., 2016; Grief and Miller, 2017; Wirtz et al., 2018; Franco-Paredes et al., 2020). In Spain, a study of immigrants showed that two-thirds suffered STD, including adolescents (Pérez-Morente et al., 2019), while a second one on homeless persons presenting themselves in hospitals revealed that they die 23–24 years earlier than the general population, and that infectious diseases were the second highest cause of death (Tornero et al., 2016). Additional factors that contribute to the high incidence of infectious diseases are low access to healthcare facilities and little information about proper prevention practices. There is no doubt that global interventions on specific diseases have a significant effect in reducing illness prevalence, particularly in the field of infectious diseases. However, it seems to be clear that the impact of many general approaches, without adequate local financial and human resources, may be limited (Beaman et al., 2018). Broadening the concept of health systems as defined by the World Health Organization (WHO, 2007), university members, teachers, and students together with off-campus partners could collaborate to promote heath at local level through well addressed interventions.

The methodologies used for teaching microbiology and infectious diseases in higher education have changed over the past few decades. Several initiatives that promote active student participation, rather than passive attendance at theoretical lecturers, such as clinical-case analysis, project-orientated laboratory work, external stage, or clinical practice (Desoubeaux et al., 2014; Horak et al., 2015; Merkel, 2016) have been designed with genuine student-centered learning objectives. Service-Learning (S-L) is a form of experiential education in which students engage in activities that address human and community needs together with structured opportunities intentionally designed to promote student learning (Jacoby, 1996). Learning objectives should be clearly connected to student curricula, and service activities should focus on real problems in the community. Beyond the acquisition of specific knowledge, additional benefits for students include achieving general competencies and skills that are more difficult to gain with traditional lecturer-centered methodologies, such as team working, decision-making, critical thinking, practical use of learned concepts, and even professional practice in attending actual problems of the society. Moreover, S-L constitutes an opportunity to contribute to the social function of the University, the others being education, research, and innovation (Klemenčič et al., 2020). In this sense, social responsibility can be open to general problems of society or focused on local community interests, or, moreover, specifically addressed to disadvantaged persons outside the university campus. Social justice can be defined as the objective of creating a fair and equal society in which every individual matters (Park, 2007), and some authors described that as a result of S-L experiences, students can make positive changes toward social justice and equality of opportunities (Einfield and Collins, 2008). Specifically, certain S-L projects focused on disadvantaged persons could be considered to have a social justice orientation (Lucman, 2020).

Service-Learning is more commonly used in education or social sciences, followed by healthcare areas (Stewart and Wubbena, 2014; Opazo et al., 2016; Dombrowsky et al., 2019), and less extended in basic or applied sciences (Santas, 2009; Begley, 2013). Recently, interesting S-L experiences related to microbiology courses have been described in several universities (reviewed by Webb, 2017) and many of them are based on teaching general microbiology to elementary or secondary pupils and the general population through hands-on demonstrations or small research projects (Abrahamsen, 2004; Vrentas et al., 2011; Mika et al., 2012; Webb, 2016; Valderrama et al., 2018; Groot et al., 2019).

A key step in designing S-L projects is evaluating resources, in terms of materials, experience, and time of teachers and potential students. After a pilot experience on the use of cinema in a clinical microbiology and parasitology course (García-Esteban, 2014), the Faculty of Biology, Universidad Complutense de Madrid, created a collection of more than 60 commercial films related to infectious diseases, and some lecturers have introduced film forum sessions in their classes. The utility of cinema as a teaching methodology is well known, and it has been used at different educational levels or for scientific divulgation to the general population (Darbyshire and Baker, 2012). Inspired by some experiences of Public Health S-L with a social justice orientation (Larios-Sanz et al., 2011; Behar-Horenstein et al., 2015; Sabo et al., 2015), the idea of using films for offering information on disease prevention seemed to be an appropriate framework for an S-L project. In addition to healthcare, contact and company are significant needs of disadvantaged populations that could be attended through cinema activities as well.

In this work, we describe the implementation of an S-L program, based on microbiology and infectious diseases at Universidad Complutense de Madrid, Spain, with students from Bachelor Degrees in Biology, Biochemistry, and Pharmacy. Our project, named *Movies and Infectious Diseases*, was incorporated in an initiative to promote S-L in higher education, together with the six public universities in the Madrid region and with Madrid City Council, with the objective of engaging university students with the demands of the local environment.

MATERIALS AND METHODS

A full development of an S-L project includes: (1) detecting a necessity in the community, (2) planning activities to attend to this need, (3) carrying out the activities, (4) evaluation and assessment, and (5) celebration and diffusion. Prior to designing the activities, the objectives of learning and service should be clearly defined, and notably, the learning objectives should be linked to student curricula. In addition, tutor abilities and

formation, as well as university resources, need to be considered. The organization and activities of the project are described in the next sections.

Detection of Community Needs and Targeted Populations

Increased exposure to pathogen transmission and lack of proper information about infectious diseases and prevention, as well as a need of attention and company, were detected as necessities in some groups of people in disadvantaged socioeconomic conditions and/or suffering social exclusion in Madrid, Spain, region. The targeted populations included the homeless, prisoners, drug addicts, immigrants, and teenagers from marginal areas.

Planification of Activity

Potential Students and Curricular Links

The project was offered to undergraduate students of Bachelor Degrees in Biology, Biochemistry, and Pharmacy, in which they follow courses in Microbiology, Clinical Microbiology, Infectious Diseases, or Public Health. It was a voluntary activity, and the students could obtain optional academic credits.

Service-Learning Team and Resources

A total of 13 university members participated in the project along two editions (two academic years): four faculty lecturers, two hospital microbiologists, one postdoctoral associate researcher, three doctoral students, two young alumni, and one undergraduate. Ten social centers, coordinated by four non-governmental organizations (NGO), collaborated as community partners. Funding was obtained from the university through Innovation and Service-Learning Calls.

A collection of commercial films that include one or several infectious diseases or related subjects was available through the Universidad Complutense library. When new films were needed, bibliographic cinema resources were used (*Journal of Medicine and Movies*, Universidad de Salamanca, Spain; *NoticiaSEM*, Spanish Society for Microbiology; *Internet Movie Database*, iMDB) and films were obtained through suitable online resources (Amazon Prime, Filmin, HBO, and Netflix). A list of selected films used in the project is provided as **Supplementary Material**.

Learning and Service Objectives

Based on the principle that educational programs should be student-centered and following main S-L focuses, in the first place, learning and service objectives were designed and, in the second place, a set of activities was proposed to achieve those objectives. **Table 1** summarizes the expected learning outcomes and the service aims related to the main activities of the project.

Action

The activities along an academic year are presented in **Figure 1**, and the description in the text will follow the numbers used in the diagram.

TABLE 1 | Learning objectives for undergraduate students related to programed project activities and service objectives addressed to target population.

Learning objectives	Main activity linked	Service objectives
Specific knowledge – Infectious diseases and their prevention and control	– Formative sessions – Preparation of scientific information and documents	Information about infectious diseases and their prevention to disadvantaged persons
Scientific skills – Search for scientific information – Critical analysis – Elaboration of scientific documents – Oral expression, divulgation	– Analysis of films – Preparation of materials for the activity – Explanatory sessions at social centers – Debate within the group – Participation in conferences or divulgation events	
General competencies – Team work and coordination – Task responsibility, self-independent work – Adaptation to new situations	– All – Coordination of team activities and with social centers	Company and dialog
Social and community engagement	– Detection of community needs – Visits to social centers	

Presentation, Student Recruitment, and Group Organization

The project was introduced to the undergraduates by lecturers during the first weeks of the academic course. Volunteers were organized into groups of four to five members, supervised by two tutors, and a social center was assigned to each group. The teams had a start-up meeting for self-organizing and assigning roles. Although the members participated in all the stages, one person was responsible for streamlining each activity: (a) team coordination and communication with tutors and community partner, (b) preparation of scientific and divulgation materials, (c) film selection and analysis, and (d) surveys undertaking and celebrations. Tutors and undergraduates worked at the same level with a non-hierarchical, horizontal, coordination.

Formative Sessions

The students received formative talks, organized in a relaxed and participative format, about the main subjects of the project: (1) infectious diseases, with a clinical microbiology analyst, (2) community needs and social exclusion, conducted by a NGO coordinator, and (3) cinema and medicine, accompanied by a filmmaker.

Teamwork and Visits to Community Partner Centers

Each team visited their assigned social center on three occasions and worked at the university to prepare the sessions. At all times, the university students were accompanied by the academic tutors and by the coordinators of the centers, following the recommended access procedures. Previously, the coordinator

FIGURE 1 | Timeline of *Movies and Infectious Diseases* Serving-Learning (S-L) project at Universidad Complutense de Madrid, Spain, along a single academic year.

student and tutors had an initial visit to know about the characteristics of the center and persons attended.

In session 1, the students conducted a dialog with the group in order to detect their interests and needs. They suggested diseases, posed, or answered questions, and finally selected one infectious illness among the different suggestions. When back in the university, the students searched for films related to the selected disease, focusing on the adequacy for the social center. Aspects such as duration, drama or action, or how the illness was treated in the film were discussed in order to select the most suitable one. Next, they critically analyzed the film, investigated the infectious disease, summarized the results in a common template, and prepared divulgation materials. A question–answer session was organized for which the students prepared putative questions that could be asked by participating persons and practised answering these using clear and correct, non-technical, scientific language.

In session 2, the film was projected in the social center, with popcorn and refreshments. Afterward, a relaxed and participative dialog was opened with the aim of resolving doubts and reinforcing key messages about infectious illnesses and prevention.

During session 3, the last one in the social centers, a voluntary survey was presented to participants, during a small celebration party, where the colloquium about infectious illnesses would continue.

Evaluation and Assessment

Assessment of the project and activities was performed using post-service surveys administered anonymously, with a Likert response 1–5 scale (1, very low/strongly disagree; 5, very high/strongly agree). All the university students and tutors completed a set of 60 questions, prepared with an online Google Forms tool, that were organized into six sections: (1) scientific competences and learning, (2) competence and skill acquisition, (3) service and social consciousness, (4) S-L methodology, (5) reasons for participating, and (6) activities and general satisfaction. For the social centers, a brief survey was completed on a voluntary basis by persons who attended and coordinators, with questions about the activity, film projection, or explanations of the students. All the surveys included blank spaces for free redaction to collect qualitative impressions, where "the best" or "the worst" of the project could be pointed out. At the end of the project, those students who wanted

to obtain optional academic credits elaborated a three-page reflection paper.

Statistical analysis of the results was performed using the GraphPad Prism Software (version 7). The non-parametric Mann–Whitney or Kruskal–Wallis tests were used for comparisons between two or more groups. For multiple comparisons between groups, Dunn's test was performed. For this, p values < 0.05 were considered statistically significant.

Celebration and Diffusion

A closing event was organized at the university with all the students, tutors, and coordinators of the community partner centers where the groups of students explained their work using a poster presentation. Additionally, supervised by their tutors, some students presented their S-L experience to scientific or divulgation conferences.

RESULTS

Participants and Academic Activities of the S-L Project

A total of 58 voluntary students participated in the project along two academic years: Biology, 4th year = 16; Biochemistry, 3rd year = 28; Biomedical Engineering, 2nd year = 1; Pharmacy 2nd year = 11; and Pharmacy, 5th year = 2. They were organized in multidisciplinary teams of different courses and years, which prompted coordination and collaborative learning.

In accordance with the characteristics and interests of the group of persons at the social centers, one or more infectious illnesses, or related aspects, as well as a suitable film were selected by students (**Table 2**).

The results of the analysis of the films were presented in two types of files: (A) *Scientific card*, which included 1. Name of the disease, 2. Causal agent/s, 3. Epidemiology (transmission, incidence, prevalence), 4. Symptomatology, 5. Diagnosis, 6. Treatment, 7. Prevention, including vaccination; and (B) *Cinematographic card*, with all the sequences related with the illness (minutes and seconds recorded) grouped in the same sections as the scientific document and including an analysis of scientific errors, when applicable. These detailed documents were used by the students as supporting material for the colloquium that was undertaken after the film projection in the social centers. Additionally, the students prepared a range of divulgation materials, such as brochures, posters, photographs or pictures, sanitary resources, and short videos, as well as entertaining games, such as Trivial or Bingo games, to accompany the discussion sessions.

TABLE 2 | Selected films about infectious diseases used in the Service-Learning (S-L) project.

Collective	Subject*	Selected film
Homeless, prisoners	STDs, AIDS	*Boys on the side*, 1995 *Dallas Buyers Club*, 2013 *La vida alegre*, 1987 *Philadelphia*, 1993 *Yesterday*, 2004
Homeless	Vaccines	*I am legend*, 2007
Teenagers, immigrants	Pandemic	*Contagion,* 2011 *World War Z*, 2013
Homeless	VBD	*Der Medicus*, 2013

**STDs, sexually transmitted diseases; AIDS, acquired immune deficiency syndrome; VBD, vector-borne diseases.*

Assessment of Utility of the S-L Strategy on Students and Community

Significant results from the post-experience surveys are presented in **Figure 2** for students (n = 29) and tutors (n = 13). The data of the two editions of the project are included and analysed together. **Figure 3** shows the results for community partners (persons attended, n = 54; social centers coordinators, n = 7) and correspond only to the first edition, as the visits to the social centers were not completed in the second edition of the project (2020) due to restrictions on movement and meetings arising from the coronavirus (COVID-19) pandemic. Mean values out of 5 and standard deviations were calculated from the 1–5 options of the scale.

Active Learning

According to the results of students and tutors' surveys, the benefits of the S-L experience were centered around acquiring or reinforcing specific knowledge about infectious diseases (4.4/5) and competences in public health (4.4/5) or science dissemination (4.6/5) (**Figure 2A**). In addition, improvement in personnel and scientific skills was also recognized (**Figure 2B**), i.e., teamwork and coordination (4.3 and 4.2 over 5, respectively), discussion of ideas and critical analysis (4.2/5), and preparation of divulgation materials (4.4/5). The evaluation of the same aspects made by the tutors rendered similar results, with no significant differences encountered (**Figures 2A,B**). Individual investigation about pathogens and diseases, film analysis and group discussions, and answering questions or explaining microbiology to non-specialized people were highlighted by students in the spaces for free writing of the surveys, as the best activities for learning. Interaction with team partners, including tutors and colleagues, was also considered as adequate for learning and preparation for future professional work (mean value 4,3/5, data not shown in **Figure 2**). Most of the participants thought that, although sometimes it was difficult to coordinate different timetables and personal schedules, the heterogeneity of team members was enriching as they could learn from each other (written opinions from surveys).

Serving Disadvantaged Persons

Evaluation of service satisfaction was achieved through a survey of community partners. The results (**Figure 3**) showed high scores for the utility of S-L activities for understanding and acquiring knowledge about infections and how to prevent them (mean of three items for attended persons and coordinators, 4.5/5), thanks to the students' preparation and adequate answers to the questions asked

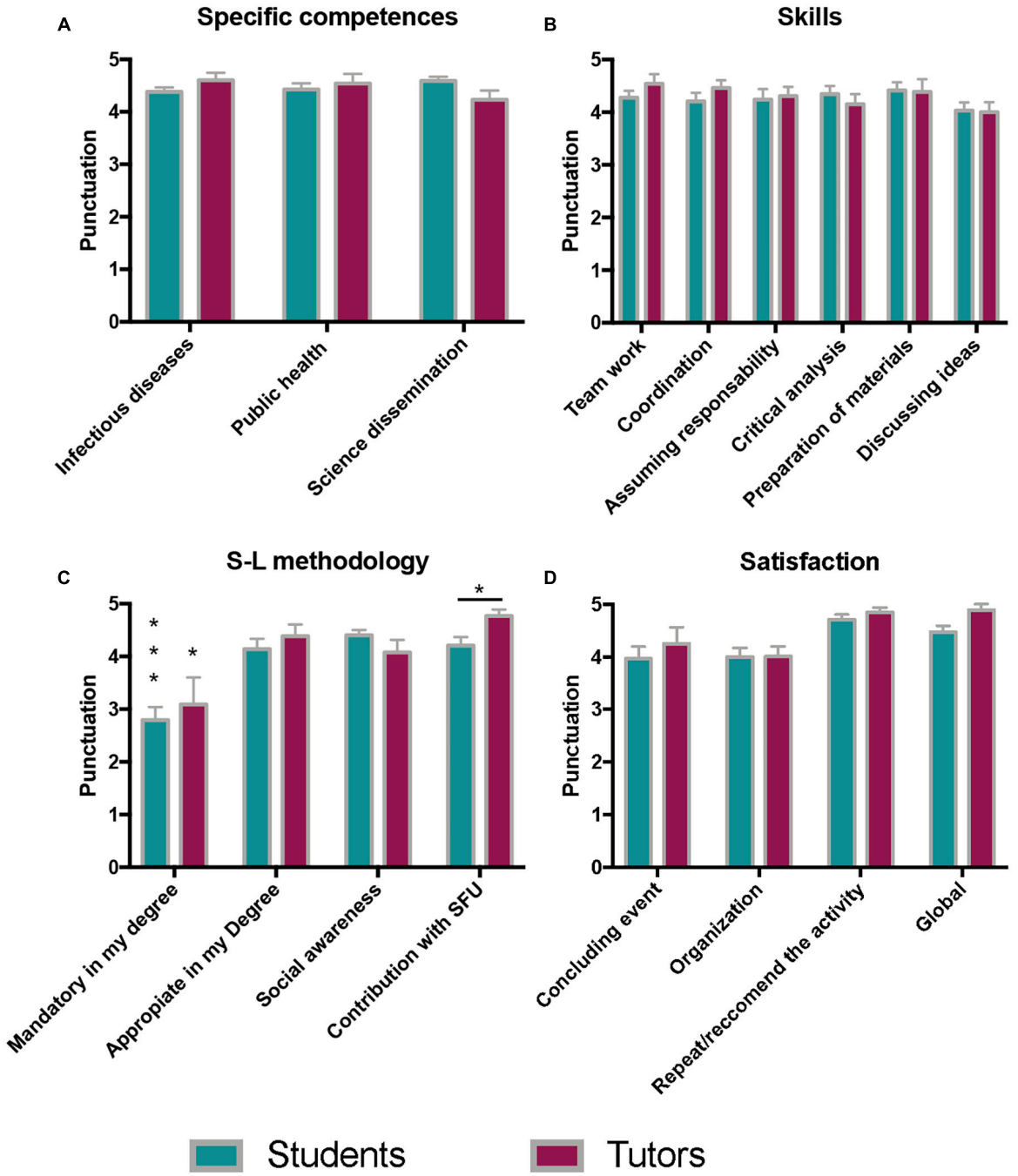

FIGURE 2 | Selected survey results from undergraduate students ($n = 29$) and tutors ($n = 13$) involved in *Movies and Infectious Diseases* S-L project at Universidad Complutense de Madrid, Spain, after 2 years of experience. **(A)** Scientific competencies and learning. **(B)** General skills acquired during the activities. **(C)** Adequacy and utility of the S-L methodology, **(D)** Satisfaction with the experience and activities. All data are expressed as mean and standard deviation on a 1–5 scale (1, very low/strongly disagree; 5, very high/strongly agree). Significant differences between the marked groups and the remaining groups are highlighted with asterisks ($^*p < 0.05$; $^{***}p < 0.0005$).

by the collectives (collective 4.9/5, coordinators, 4.7/5). In the written opinions, the disadvantaged persons appreciated most profoundly the company, respect, attention, and equal treatment of the students and tutors. The data presented in **Figure 3** correspond only to the first edition, as the visits to the social centers were not completed in the second edition of the project in 2020, as previously mentioned. Nevertheless, the coordinators of NGOs, who participated in the online closing event where the students presented their work (9, **Figure 1**), highlighted the usefulness of the activity and the

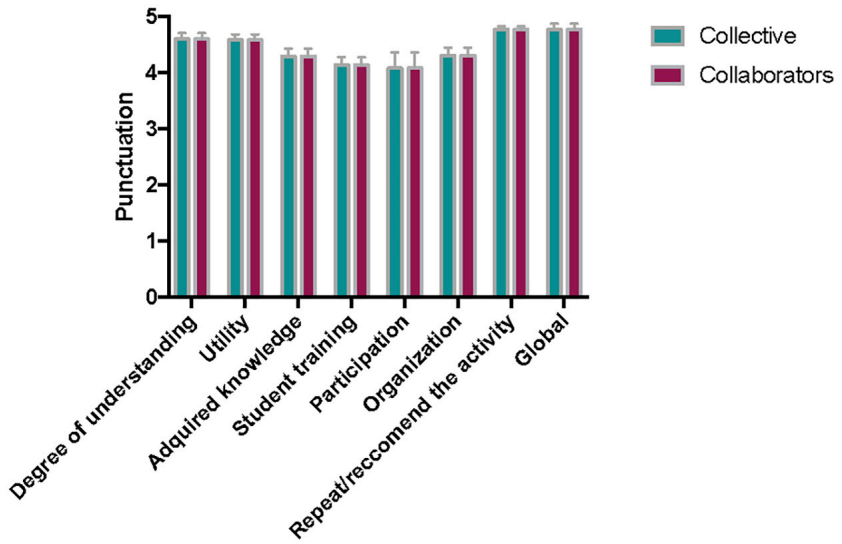

FIGURE 3 | Selected survey results from community partners (collective, $n = 54$; collaborators, $n = 7$) involved in *Movies and Infectious diseases* S-L project at Universidad Complutense de Madrid, Spain. All data are expressed as mean and standard deviation on a 1–5 scale (1, very low/strongly disagree; 5, very high/strongly agree).

materials prepared by the students for divulgation at their centers (data not shown).

Service-Learning as an Efficient Educational Methodology at the University

The motivations of the university students for choosing the *Movies and Infectious Diseases* S-L project were (in order of punctuation) social involvement (4.6/5), the possibility to acquire general competences and skills (4.5/5), curiosity (4.2/5), and strengthening of academic knowledge of clinical microbiology (4.1/5). In comparison, earning elective credits and occupying their time were not important motivations (2.7 and 2.2, respectively) with significant differences ($p < 0.05$ and $p < 0.0005$, respectively).

Undergraduates and tutors thought that S-L methodology was adequate for their degrees, increased their social awareness, and contributed to the social responsibility of the University (4.0–4.8). Nevertheless, although many of them described the activity as enriching and directly applicable to their future work in clinical microbiology, public health, or related areas (written opinions in surveys), most of them considered that S-L should not be mandatory within microbiology courses (mean value of teachers and students, 2.8/5 and $p < 0.05$). The data of S-L methodology are shown in **Figure 2C**.

Finally, global satisfaction of the participants was used to measure the success of the project and, notably, high scores were obtained (4.5/5 students and 4.6/5 tutors, in **Figure 2D**; 4.8 collective and 4.6 coordinators in **Figure 3**). Most of the participants would join again and recommend the S-L project to companions (4.8 and 4.7 out of 5 for students and teachers, respectively, **Figure 2D**; 4.8 for persons and coordinators in social centers, **Figure 3**).

DISCUSSION

Service-Learning initiatives specifically focused on clinical microbiology are usually included as a part of Public Health actions for Nursing or Medicine courses (Larios-Sanz et al., 2011; Abu-Shakra, 2012; Cain, 2013; Stewart and Wubbena, 2014), but they are less used in Bachelor Degrees in Sciences. The S-L program described in this work was developed with students and university tutors of Biology, Biochemistry, and Pharmacy, as these degrees include courses on Clinical Microbiology, and this would indicate the usefulness of this type of S-L programs in different university careers. Similarly, another S-L experience was carried out in Universidad Complutense de Madrid, based on antibiotic resistance, in which the Faculties of Biology, Pharmacy and Veterinary were involved (Valderrama et al., 2018). To our knowledge, cinema had not been applied in S-L experiences in clinical microbiology (Webb, 2017), although it is frequently used as a tool for teaching infectious diseases (García-Sánchez et al., 2002). The results of our project clearly showed the utility of commercial films in the student learning process at the university level and the importance of attending to community needs.

Concerning the learning objectives, specific knowledge about infectious diseases and public health were significant achievements according to the results of the surveys for students and tutors. As only post-experience surveys were carried out on the students, no data were available to compare scientific knowledge before and after the activity. However, in some studies, it was found that final grades of students who participated in an S-L project and those who followed traditional lecturers in clinical microbiology were similar (Cain, 2013). Most frequently, specific learning outcomes through S-L are not quantified in terms of global academic marks but the progress is appreciated by the students themselves and their teachers (Abu-Shakra,

2012; Valderrama et al., 2018). The data from the student surveys did not show a significant difference between acquiring new concepts and reinforcing previous ones, probably because most of the participants had studied a clinical microbiology course before joining the project and they had already acquired some background knowledge. Rather than being a pitfall, it is considered that strengthening of learned concepts by applying them to real situations is one the goals of S-L (Cain, 2013). Critical analysis of the films was highlighted in the surveys as a very adequate method for learning, as has been previously described when using cinema to other health areas (Weerts, 2005). Nevertheless, a literature review of the use of commercial films as a teaching resource for health sciences students concluded that there are few studies that quantify the increase in learning to clearly demonstrate the usefulness of the methodology, beyond the subjective perception of students and lecturers (Díaz et al., 2015).

In our project, students from different degrees and academic levels worked in mixed groups that included juniors (2nd year) and some seniors (4th or 5th year) and the heterogeneity was pointed out in the surveys as an ideal form of learning from teammates. This benefit was previously observed in several collaborative learning courses in microbiology, where junior students were helped by more experienced ones (Rutherford, 2015; Hou et al., 2018). Moreover, it has been described that the undergraduates do not need to have a high level of specific knowledge to participate, although they will require more supervision by their tutors (Dombrowsky et al., 2019).

Salient benefits of S-L on students' academic progress are related to skills acquisition, as can be concluded from our results. Competence on explaining microbiology to non-experienced people, answering questions, and clarifying doubts about infections, transmission of pathogens, or prevention procedures can be highlighted. In the same sense, learning by teaching others has been described as an efficient methodology in microbiology and other disciplines (Rutherford, 2015; Webb, 2016, 2017). Additionally, team-working ability, coordination, assuming responsibilities or leadership, and problem solving and adaptation were freely expressed by the students in their surveys as significant goals, and similar results have been described for other S-L programs (Opazo et al., 2016; Valderrama et al., 2018).

Assessing Service-Learning projects should not only focus on student learning, and the community service must be also evaluated. The high level of satisfaction of social partners confirmed the usefulness of the *Movies and Infectious Diseases* program for addressing real needs of outside-campus communities. Although it might be difficult to assess service outcomes through quantitative surveys, it is recognized that the results obtained from post-experience reports, oral opinions, and group discussions are also valuable (Holland, 2001). In our S-L experience, social awareness and engagement were the main motivations and satisfaction elements for students and tutors, as is characteristically described for this kind of projects (Bringle and Clayton, 2012). As for the students, the most repeated positive impressions (marked as "the best" in the surveys) were "*discovering social situations unknown until then*," "*meeting disadvantaged persons*," and "*sharing their scientific knowledge with others in need*."

In summary, the results of the program "*Movies and Infectious Diseases*" illustrate that S-L fulfils the objective of active learning of university students on clinical microbiology courses while attending community needs, i.e., helping to prevent infections to disadvantaged persons. We understand that the S-L program implemented is an excellent strategy from an academic point of view, which promotes not only scientific knowledge but, almost most importantly, critical thinking, teamwork, and responsibility. Moreover, the connection with the real needs of the community prompted personal and group engagement in social justice in both university students and tutors.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics and Deontology Committee. University Complutense of Madrid (signed by F. J. M. Fernández Vallina, President of the Committee). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

MJV: conceptualization. PÁ, EC, ED, MTG, NL-E, ML, CM, MLM, AR-G, IR-A, CLU, MJV, and MVV: experimental procedures. ML, NL-E, and MJV: data analysis and interpretation. MJV: the manuscript preparation. All authors have read an agreed this version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.589401/full#supplementary-material

REFERENCES

Abrahamsen, L. (2004). Learning partnerships between undergraduate biology students and younger learners. *Microbiol. Educ.* 5, 21–29. doi: 10.1128/jmbe.v5.74

Abu-Shakra, A. (2012). Including a service learning educational research project in a biology course-II: assessing community awareness of legionnaires' disease? *Euro. J. Educ. Res.* 1, 271–282. doi: 10.12973/eu-jer.1.3.271

Beaman, A., Asano, R., Sibbritt, D., Newton, P. J., and Davidson, P. M. (2018). Global service learning and health systems strengthening: an integrative literature review. *Heliyon* 4:e00713.

Begley, G. S. (2013). Making connections: service-learning in introductory cell and molecular biology. *J. Microbiol. Biol. Educ.* 14, 213–220. doi: 10.1128/jmbe.v14i2.596

Behar-Horenstein, L. S., Feng, X., Roberts, K. W., Gibbs, M., Catalanotto, F. A., and Hudson-Vassell, C. M. (2015). Developing dental students' awareness of health care disparities and desire to serve vulnerable populations through service-learning. *J. Dental Educ.* 79, 1189–1200. doi: 10.1002/j.0022-0337.2015.79.10.tb06012.x

Bringle, R. G., and Clayton, P. H. (2012). "Civic education through service learning: what, how, and why?," in *Higher Education and Civic Engagement Comparative Perspectives*, eds L. McIlrath, A. Lyons, and R. Munck (New York, NY: Palgrave Macmillan). doi: 10.1057/9781137074829_7

Cain, D. M. (2013). Impact of a service-learning project on student success in allied health microbiology course. *J. Microbiol. Biol. Educ.* 14, 129–130. doi: 10.1128/jmbe.v14i1.541

Darbyshire, D., and Baker, P. (2012). A systematic review and thematic analysis of cinema in medical education. *Med. Humanit.* 38, 28–33. doi: 10.1136/medhum-2011-010026

Desoubeaux, G., Simon, E. G., Perrotin, D., and Chandenier, J. (2014). The mobile team of parasitology-mycology, a medical entity for educational purposes to serve sick patients. *J. Mycol. Med.* 24, 144–151.

Díaz, M., Icart, M. T., and López, M. C. (2015). Literature review: use of commercial films as a teaching resource for health sciences students. *Nurse Educ. Today* 36, 264–267. doi: 10.1016/j.nedt.2015.10.002

Dombrowsky, T., Gustafson, K., and Cauble, D. (2019). Service-learning and clinical nursing education: a delphi inquiry. *J. Nurs. Educ.* 58, 381–391. doi: 10.3928/01484834-20190614-02

Einfield, A., and Collins, D. (2008). The relationships between service-learning, social justice, multicultural competence and civic engagement. *J. Coll. Stud. Dev.* 49, 95–109. doi: 10.1353/csd.2008.0017

Franco-Paredes, C., Jankousky, K., Schultz, J., Bernfeld, J., Culle, K., Quan, N. G., et al. (2020). COVID-19 in jails and prisons: a neglected infection in a marginalized population. *PLoS Negl. Trop. Dis.* 4:e0008409. doi: 10.1371/journal.pntd.0008409

García-Esteban, M. T. (2014). *Enfermedades Infecciosas: Cámaras Y ... ¡Acción!*. Madrid: Universidad Complutense de Madrid.

García-Sánchez, J. L., Fresnadillo, M. J., and García-Sánchez, E. (2002). Movies as a teaching resource for infectious diseases and clinical microbiology. *Enferm. Infecc. Microbiol. Clin.* 20, 403–406.

Grief, S. N., and Miller, J. P. (2017). Infectious disease issues in underserved populations. *Prim. Care Clin. Office Pract.* 44, 67–85. doi: 10.1016/j.pop.2016.09.011

Groot, P. W. J., Fernandez-Pereira, J., Sabariegos, R., Clemente-Casares, P., Parra-Martínez, J., Cid, V. J., et al. (2019). Optimizing small world initiative service learning by focusing on antibiotics-producing actinomycetes from soil. *FEMS Microbiol. Lett.* 366:fnaa019. doi: 10.1093/femsle/fnaa019

Holland, B. A. (2001). A comprehensive model for assessing service-learning and community-university partnerships. *New Dir. High. Educ.* 114, 51–60. doi: 10.1002/he.13

Horak, R., Merkel, S., and Chang, A. (2015). Developing research-based undergraduate microbiology curricula. *Microbe* 10, 329–334. doi: 10.1128/microbe.10.329.1

Hou, Y. J., Liu, W. Y., Lin, Y. H., Lien, H. Y., Wong, A. M. K., and Chen, C. M. (2018). A pediatric service-learning program in physical therapy education. *Pediatr. Phys. Ther.* 30, 149–154. doi: 10.1097/PEP.0000000000000498

Jacoby, B. (1996). *Service-Learning in Higher Education: Concepts and Practices*. San Francisco, CA: Jossey-Bass.

Klemenčič, M., Pupinis, M., and Kirdulytė, G. (2020). *'Mapping and Analysis of Student-Centred Learning and Teaching Practices: Usable Knowledge to Support More Inclusive, High-Quality Higher Education'*, NESET Report. Luxembourg: Publications Office of the European Union. doi: 10.2766/67668

Larios-Sanz, M., Simmons, A. D., Bagnall, R. A., and Rosell, R. C. (2011). Implementation of a service-learning module in medical microbiology and cell biology classes at an undergraduate Liberal Arts University. *J. Microbiol. Biol. Educ.* 12, 29–37. doi: 10.1128/jmbe.v12i1.274

Lucman, S. T. (2020). Developing social justice skill through community service-learning in high school social studies. *J. Soc. Stud.* 81:2.

Merkel, S. M. (2016). American society for microbiology resources in support of an evidence-based approach to teaching microbiology. *FEMS Microbiol. Lett.* 363:fnw172.

Mika, K. B., Lin, T. Y., Ferreira, M., Lacson, J., Lee, C. M., Lin, C.-C., et al. (2012). Incorporating service learning in traditionally lecture-based environmental engineering courses through researching bacterial contamination at a local beach. *Global J. Eng. Educ.* 14, 155–162.

Opazo, H., Aramburuzabala, P., and Cerrillo, R. (2016). A review of the situation of service-learning in higher education in Spain. *Asia Pac. J. Coop. Educ.* 17, 75–91. doi: 10.13140/RG.2.1.3046.1841

Park, C. (2007). *A Dictionary on Environment and Conservation*. New York, NY: Oxford University Press.

Pérez-Morente, M. A., Campos-Escudero, A., Sánchez-Ocón, M. T., and Hueso-Montoro, C. (2019). Características sociodemográficas, indicadores de riesgo y atención sanitaria en relación a infecciones de transmisión sexual en población inmigrante de Granada. *Rev. Esp. Salud Pública.* 93:e201912102.

Raoult, D., Foucault, C., and Brouqui, P. (2001). Infections in the homeless. *Lancet Infect. Dis.* 1, 77–84. doi: 10.1016/S1473-3099(01)00062-7

Rudge, S., Webster, I., and van Beek, I. (2018). Infectious diseases in homeless people. *NSW Public Health Bull.* 19, 76–77. doi: 10.1071/NB07128

Rutherford, S. (2015). E pluribus unum: the potential of collaborative learning to enhance microbiology teaching in higher education. *FEMS Microbiol. Lett.* 362:fnv191. doi: 10.1093/femsle/fnv191

Sabo, S., de Zapien, J., Teufel-Shone, N., Rosales, C., Bergsma, L., and Taren, D. (2015). Service learning: a vehicle for building health equity and eliminating health disparities. *Am. J. Public Health* 105, 38–43. doi: 10.2105/AJPH.2014.302364

Santas, A. J. (2009). Reciprocity within biochemistry and biology service-learning. *Biochem. Mol. Biol. Educ.* 37, 143–151. doi: 10.1002/bmb.20291

Sivic, S., Huremovic, A., and Djerzic, H. (2013). Social exclusion as a determining health factor of the Roma population. *Med. Arh.* 67, 60–62. doi: 10.5455/medarh.2013.67.60-62

Stewart, T., and Wubbena, Z. (2014). An overview of infusing service-learning in medical education. *Int. J. Med. Educ.* 5, 147–156.

Tornero, S., Fernández, A., and Charris, L. (2016). Características de los ingresos hospitalarios de las personas sin hogar en Sevilla. *Rev. Esp. Salud Pública.* 90, 1–11.

Tsai, J., and Wilson, M. (2020). COVID-19: a potential public health problem for homeless populations. *Lancet Public Health* 5, e186–e187. doi: 10.1016/S2468-2667(20)30053-0

Valderrama, M. J., González-Zorn, B., Calvo, P., Díez-Orejas, R., Fernández-Acero, T., Gil-Serna, J., et al. (2018). Educating in antimicrobial

resistance awareness: adaptation of the small world initiative program to service-learning. *FEMS Microbiol. Lett.* 365:fny161. doi: 10.1093/femsle/fny161

Vrentas, C. E., Zinnen, T., and Huebert Lima, D. J. (2011). The microsafari: a journey into microbiology, an expedition into engagement. *J. Microbiol. Biol. Educ.* 12, 61–63. doi: 10.1128/jmbe.v12i1.265

Webb, G. (2016). Learning through teaching: a microbiology service-learning experience. *J. Microbiol. Biol. Educ.* 17, 86–89. doi: 10.1128/jmbe.v17i1.997

Webb, G. (2017). A review of microbiology service learning. *FEMS Microbiol. Lett.* 364:fnx032. doi: 10.1093/femsle/fnx032

Weerts, S. (2005). Use of films to teach critical thinking. *J. Nutr. Educ. Behav.* 37, 100–101. doi: 10.1016/s1499-4046(06)60025-0

Wirtz, A. L., Yeh, P. T., Flath, N. L., Beyrer, C., and Dolan, K. (2018). HIV and viral hepatitis among imprisoned key populations. *Epidemiol. Rev.* 40, 12–26. doi: 10.1093/epirev/mxy003

WHO (2007). *Everybody's Business: Strengthening Health Systems to Improve Health Outcomes: WHO's Framework For Action*. Geneva: World Health Organization.

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Student Outcomes From a Large-Enrollment Introductory Course-Based Undergraduate Research Experience on Soil Microbiomes

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In recent years, national reports have called for undergraduate laboratory education that engages students in authentic research experiences. As a result, a number of course-based undergraduate research experiences (CUREs) have been developed in biological sciences and some specifically in microbiology. Students benefit from CUREs much like in traditional mentored research experiences, where students carry out independent projects in faculty laboratories. These benefits include increased self-efficacy in research skills, enhanced identification as scientists, and higher graduation rates in science, technology, engineering, and mathematics majors. Because mentored research experiences are not readily available to every student, CUREs represent a potential mechanism to democratize the research experience by providing such opportunities to all students. However, many of existing CUREs described in the literature are designed for advanced undergraduates or are limited to a small number of students. Here, we report student outcomes from a large-enrollment introductory CURE on soil microbiomes that engages students in a real-world context with microbiology. In pre- and post-course surveys, students reported significant gains in self-efficacy on a number of research skills. These results are triangulated with post-course survey data on project ownership, sense of community, and CURE design elements such as collaboration, iteration, discovery, and relevance.

Keywords: course-based undergraduate research experience, introductory biology, laboratory education, large enrollment, soil microbiome

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INTRODUCTION

Research and laboratory experiences are important aspects of undergraduate education in biological sciences. In the past few decades, many national reports have called for the incorporation of research experiences into undergraduate education. Following broad calls in Science for All Americans (American Association for the Advancement of Science [AAAS], 1989) and Reinventing Undergraduate Education (Boyer Commission, 1998), more specific recommendations in

biological sciences began to emerge: engaging students in the excitement of discoveries (National Research Council [NRC], 2003) and incorporating research experiences into laboratory courses in the first 2 years of the undergraduate curriculum (President's Council of Advisors on Science, and Technology [PCAST], 2012). Recently, calls for transforming undergraduate education more broadly in science, technology, engineering, and mathematics (STEM) have increasingly focused on students across diverse educational contexts (National Academy of Sciences [NAS] et al. , 2011; National Academies of Sciences, Engineering, and Medicine [NASEM], 2016).

Research experiences lead to improved outcomes for undergraduate students in many domains, such as disciplinary knowledge and competencies, professional and personal skills, identification as scientists, and persistence and time to degree in STEM (Hunter et al., 2006; Kinkel and Henke, 2006; Desai et al., 2008; Edwards et al., 2011; President's Council of Advisors on Science, and Technology [PCAST], 2012; Horowitz and Christopher, 2013; Palmer et al., 2015). These outcomes are disproportionately beneficial for students from minoritized demographics, such as women and underrepresented minorities (Summers and Hrabowski, 2006; Summers, 2011; President's Council of Advisors on Science, and Technology [PCAST], 2012). The Association of American Colleges and Universities considers undergraduate research experiences a high-impact educational practice that has been "widely tested and shown to be beneficial of college students from many backgrounds" (Kuh, 2008).

Mentored research experiences are available to a limited number of students. Especially at large public research universities, it is logistically infeasible for every undergraduate student to engage in mentored research experiences in faculty laboratories, simply given the student-to-faculty ratio. For example, at our institution, there are over 5,000 undergraduates majoring in biological sciences, with only about 100 faculty in the Division of Biological Sciences. Course-based undergraduate research experiences (CUREs) can be designed as part of the standard undergraduate laboratory curriculum, thus serving as a mechanism to make research experiences accessible to all students (Auchincloss et al., 2014). CUREs represent a democratization of the research experience by providing such opportunities to a much larger number of students, including students who belong to minoritized groups that have been historically underrepresented in science (Bangera and Brownell, 2014).

Course-based undergraduate research experiences engage students in scientific inquiry (Buck et al., 2008; Weaver et al., 2008) and are defined by a number of design elements: utilizing scientific practices, engaging with the collaborative and iterative nature of research, and making novel discoveries with broader relevance (Brownell and Kloser, 2015; Corwin et al., 2015a). Students benefit from CUREs much like in mentored research experiences, including increased self-efficacy in research skills, enhanced identification as scientists, and higher graduation rates in STEM majors (Lopatto et al., 2008; Shapiro et al., 2015; Rodenbusch et al., 2016). Many CUREs in biological sciences have been developed in the existing literature, such as annotating genome sequences, examining abiotic and biotic factors in ecology, investigating drug resistance in proteins (Chen et al., 2005; Taylor et al., 2010; Kloser et al., 2011, 2013). Examples in microbiology include discovering antibiotics, identifying bacteriophages, examining biofilms, and synthesizing biofuels (Hanauer et al., 2006; Davis et al., 2017; Pedwell et al., 2018; Light et al., 2019). However, many of these CUREs are for advanced undergraduates (Caspers and Roberts-Kirchhoff, 2003; Taylor et al., 2010; Butler et al., 2014; Murthy et al., 2014), and some are limited to a small number of students (Kloser et al., 2011, 2013; Thompson et al., 2016; Bhatt and Challa, 2018).

Previously, we reported the design and implementation of a large-enrollment introductory CURE on soil microbiomes that engages students in a real-world context with microbiology (Lo and Mel, 2017; Lo and Mordacq, 2020). Students work in teams to collect soil samples from native and invasive plant species at a biodiversity hotspot (Myers et al., 2000), compare soil properties such as moisture and pH, characterize microbial genetic biodiversity by 16S rRNA gene sequencing, and perform colorimetric assays to determine carbon source utilization of different soil microbiomes. Student teams also develop research proposals that they present at a poster conference to compete for mock grant funding. In this paper, we describe student outcomes from this CURE, including self-efficacy on research skills, project ownership, and sense of community.

MATERIALS AND METHODS

Course Context

This study was conducted in the United States at a 4-year, public not-for-profit, and large doctoral university, described by The Carnegie Classification of Institutions of Higher Education (McCormick and Zhao, 2005) in the category of "very high research activity" and with a full-time, more selective, and higher transfer-in undergraduate profile. Human subject research was approved by the Institutional Review Board at the University of California San Diego. The CURE in this paper is part of the Introductory Biology Laboratory course at the study institution, which is a stand-alone course without prerequisites and not associated with lecture-based courses. Laboratory sections in the course meet once a week, and all learning activities are connected with the soil microbiome project in the CURE.

We define authentic research experiences in our CURE using the situated learning theory, which posits that learning takes place in the same context in which it is applied (Lave and Wenger, 1991) and as part of a community of practice (Wenger, 1999). Situated learning occurs through a process called legitimate peripheral participation (Lave and Wenger, 1991), meaning that students engage in the same tasks that scientists would do in a real research setting ("legitimate"), even though students may be performing at a less complex or sophisticated level ("peripheral"). Specifically, students collaborate in research projects that can result in novel conclusions with broader relevance, and they engage in the iterative nature of scientific inquiry (**Table 1**).

Study Samples

Pre-course surveys were given in the first 2 weeks of the quarter. Post-course surveys were administered in the last 2 weeks of the quarter prior to final examinations. In our institutional context, this was the timeframe in which the student course evaluations were also administered on campus. In the past, we found that asking students to complete surveys after final examinations resulted in very low response rates. Therefore, we opted to administer the surveys for our study at the same time as the student course evaluations.

Survey data were collected over two academic quarters. In earlier implementations of the CURE, we observed many incomplete survey responses, and students expressed dissatisfaction with the number of surveys in the course, suggesting respondent fatigue (Ben-Nun, 2008). Therefore, we administered different subsets of surveys across the academic year (**Table 2**). While this approach resulted in a smaller data set, which reduces statistical power, we reasoned that the rotation of surveys could potentially yield a higher response rate and more meaningful responses. Historically, the overall grade distributions of the course have remained consistent across academic quarters over the years, suggesting minimal variations in the student populations that enroll in the course in different academic quarters.

Survey Instruments

Student outcomes were measured pre- and post-course by the classroom undergraduate research experience survey (Denofrio et al., 2007). We used a modified version of the classroom undergraduate research experience survey that changed the five-point scale on self-reported post-course learning gains (1 = no gain, 5 = very large gain) to a six-point scale on pre- and post-course self-efficacy (1 = no skill, 6 = very high skill) to capture the pre-course baseline. The six-point scale was intentionally chosen to eliminate the ambiguous mid-point option in the original five-point scale, which could be interpreted as neutral or undecided, two similar but distinct constructs (Komorita, 1963; Guy and Norvell, 1977; Armstrong, 1987). These modifications were previously determined to retain high internal consistency and reliability (Mordacq et al., 2017).

We also measured student outcomes using the project ownership survey (Hanauer and Dolan, 2014) and the classroom community inventory (Rovai et al., 2004). The laboratory course assessment survey (Corwin et al., 2015b) was also administered to capture student perspectives on whether specific CURE design elements such as collaboration, iteration, discovery, and relevance were present in the course. These three surveys were administered only at the end of the course ("post-course"), as they describe student experiences within the course, and the items would not make sense at the beginning of the course ("pre-course"). For these instruments, we used the various Likert or Likert-like scales in the original literature, some of which were on a five-point scale. This is based on recommendations to allow for neutral responses instead of forced directional choices for items especially related to emotions and affect (Komorita, 1963; Guy and Norvell, 1977; Armstrong, 1987).

TABLE 1 | CURE design elements.

Design element	Course structure
Scientific practices	Students collect and analyze data to draw conclusions
Collaboration	Teams of students collaborate and share research data
Iteration	Previous results are incorporated into assignments
Discovery	Novel soil microbiome data are collected by students
Relevance	Research question is of interests to professional scientists

Specific course structure and activities were developed in alignment with the CURE design elements described in the existing literature: scientific practices, collaboration, iteration, discovery, and relevance.

TABLE 2 | Survey administration response rates.

Survey instrument	Response rate
Classroom undergraduate research experience survey	165/248 (82.1%)
Project ownership survey	203/239 (85.4%)
Classroom community inventory	165/248 (82.1%)
Laboratory course assessment survey	203/239 (85.4%)

Different subsets of surveys were administered across two academic quarters to minimize respondent fatigue.

Statistical Analysis

Descriptive statistics were calculated for all survey responses. For the Classroom Undergraduate Research Experience survey, only matched pre-and-post pairs were included for analysis. Pre- and post-course responses were compared using the Wilcoxon signed-ranked test because of the non-parametric nature of the data (Wilcoxon, 1945), and the Holm-Bonferroni correction was used to correct for multiple comparisons (Holm, 1979; Shaffer, 1995). Effect sizes were calculated using Cohen's d, which is defined as the difference between the pre- and post-course means normalized to the standard deviation from the pre-course data (Maher et al., 2013). For the items administered only post-course, analysis of variance with the Tukey's honestly significant difference (HSD) test was used to determine if responses for items within each survey construct were statistically different. All statistical analyses were performed in JMP Pro Version 13.0–16.0 or Microsoft Excel.

RESULTS

Pre- and post-course results from the classroom undergraduate research experience survey showed that students reported self-efficacy gains in 22 out of 25 items (**Table 3**). In the category of research skills, significant gains ($p < 0.05$) in self-efficacy ranged from 0.16 to 0.85 in effect size across nine out of 10 items. Writing a research proposal and reading scientific literature showed the highest gains with effect sizes of 0.85 and 0.80, respectively, which are considered large (Maher et al., 2013). This

large gain likely resulted from the course project in addition to the laboratory experiments on soil microbiomes, where student teams developed research proposals based on primary literature of interests to them. Performing computer calculations and maintaining a research notebook had effect sizes of 0.79 and 0.56, respectively, which are considered medium (Maher et al., 2013). Both were regular activities done in laboratory sections every week. Analyzing research data had a much smaller effect size of 0.24, despite also being part of the laboratory activities each week. This survey item may be less specific compared to performing computer calculations and maintaining a research notebook and thus did not resonate in students' mind as something they had done regularly in the course. Critiquing work of other students and presenting results as papers had effect sizes of 0.42 and 0.24, which are considered small (Maher et al., 2013). These activities only occurred 3–4 times throughout the quarter and thus likely resulted in the smaller effect sizes. Surprisingly, no statistical difference was observed pre- and post-course for self-efficacy in presenting a poster, even though student teams presented their research proposals as posters in a conference format as their final examination. This was likely due to the timing of the survey administration, which was completed before the week of final examinations to encourage a higher response rate.

In the category of experience with different types of research projects, significant gains ($p < 0.05$) in self-efficacy ranged from 0.17 to 0.64 in effect size across all seven items. Students reported highest gains in doing a project where no one knows the outcome, where students have some input, and where entirely designed by students with effect sizes of 0.64, 0.61, and 0.59, respectively, which are considered moderate (Maher et al., 2013). In the course, we emphasized that the soil microbiome project was original research where the students would be the first to collect and analyze their data and that no other students had previously reported the same data. The CURE aspect of the course, along with the research proposals developed by student teams, likely resulted in these moderate effect size.

In the category of general course skills, six out of eight items showed significant gains ($p < 0.05$) with effect sizes ranging from 0.20 to 0.47, which are considered small (Maher et al., 2013). Many of these items were not directly related to the CURE aspects of the course, and students would have likely reported gains in working on problems, listening to lectures, and taking tests even if they were in a lecture course or a non-CURE laboratory course. Reading a textbook and working individually showed no statistical difference pre- and post-course. These were not activities emphasized in the course, as there was minimal reading other than the laboratory manual, and students always worked in teams of laboratory experiments and their research proposals.

In terms of project ownership (**Table 4**), students reported highest post-course ratings in the items "my research project was interesting" (average ± standard deviation = 4.2 ± 0.5 on a five-point Likert scale) and "my project gave me a sense of personal achievement" (3.9 ± 0.8). Students also reported lowest post-course ratings in the item "I had a personal reason for choosing the research project" (3.1 ± 1.9). The latter result was perhaps not surprising, as the soil microbiome project was relatively structured and not chosen by individual students given the large-enrollment nature of the course.

TABLE 3 | Classroom undergraduate research experience survey.

Item	Pre	Post	*p*	ES
Research skills				
Write a research proposal	2.0 ± 0.9	2.7 ± 0.8	****	0.85
Read scientific literature	2.5 ± 0.8	3.1 ± 0.7	****	0.80
Perform computer calculations	1.6 ± 0.8	2.4 ± 0.9	****	0.79
Maintaining research notebook	3.0 ± 1.0	3.5 ± 0.8	****	0.56
Critique work of other students	2.8 ± 0.8	3.1 ± 0.7	****	0.42
Analyze research data	3.3 ± 0.7	3.5 ± 0.7	**	0.24
Present results in papers	3.1 ± 0.9	3.3 ± 0.8	**	0.24
Collect data	3.4 ± 0.7	3.5 ± 0.8	*	0.17
Present results orally	2.7 ± 0.9	2.9 ± 0.8	*	0.16
Present a poster	3.0 ± 1.0	3.0 ± 0.8	n.s.	0.05
Doing a project where . . .				
No one knows the outcome	2.0 ± 0.9	2.6 ± 0.9	****	0.64
Students have some input	2.5 ± 1.0	3.0 ± 0.8	****	0.61
Entirely designed by students	2.1 ± 0.9	2.7 ± 0.9	****	0.59
Students are responsible	3.8 ± 0.7	4.0 ± 0.7	**	0.28
Instructor knows the outcomes	3.0 ± 0.8	3.2 ± 0.8	**	0.28
Structured by the instructor	3.5 ± 0.9	3.7 ± 0.8	*	0.20
Students know the outcome	3.1 ± 0.8	3.3 ± 0.9	*	0.17
General course skills				
Work on problems	3.6 ± 0.7	4.0 ± 0.8	*	0.47
Listen to lectures	3.9 ± 0.7	4.2 ± 0.7	*	0.39
Work as a whole course	2.9 ± 0.8	3.2 ± 0.8	***	0.31
Take tests	4.1 ± 0.6	4.2 ± 0.7	*	0.27
Work in small groups	3.8 ± 0.6	3.9 ± 0.6	*	0.22
Discuss reading materials	3.4 ± 0.8	3.6 ± 0.7	*	0.20
Read textbook	3.9 ± 0.8	4.0 ± 0.8	n.s.	0.12
Work individually	3.5 ± 0.9	3.6 ± 1.0	n.s.	0.11

*Items are grouped into three categories (research skills, experiences with different types of projects, and general course skills) and ordered by effect size (ES, calculated as Cohen's d) from large to small within each category. Items are on a six-point Likert-like scale (1 = no skill, 6 = very high skill). Descriptive statistics (average ± standard deviation) are reported. Statistical differences are indicated by the following notation: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; and n.s., not significant.*

For the results from the emotions items on a five-point Likert-like scale, students reported being delighted (3.7 ± 0.8), happy (3.7 ± 1.1), and joyful (3.4 ± 1.0) more so than being amazed (3.0 ± 1.0), surprised (2.8 ± 0.9), and astonished (2.7 ± 0.9). These results were similar to those from published sources (Hanauer and Dolan, 2014), with the exception of surprised and astonished, which were positive in the original study. In our CURE, the research project compared soil properties and microbiomes associated with native and invasive plant species. While the comparison was helpful in teaching basic hypothesis testing and statistics, there was simply no reason for students to envision *a priori* which soil sample would have a higher pH or more diverse microbiome. Correspondingly, it would seem reasonable that students were not surprised or astonished.

For classroom community, students reported 3.4 ± 0.7 and 3.1 ± 0.4 (on a five-point Likert scale) for the peer support and learning support dimensions, respectively (**Table 5**). Peer

TABLE 4 | Project ownership survey.

Item	Avg	SD	A	B	C	D
Ownership						
My research project was interesting	4.2	0.5	A			
My project gave me a sense of personal achievement	4.1	0.7	A			
I was responsible for the outcomes of my research	4.0	0.7	A	B		
In my project, I actively sought advice and assistance	4.0	0.8	A	B		
I faced challenges that I managed to overcome	3.9	0.6	A	B		
My findings were important to the scientific community	3.7	0.7		B	C	
My research will help to solve a problem in the world	3.7	0.7		B	C	
My research project was exciting	3.7	0.8			C	
The research question I worked on was important to me	3.5	0.8			C	
I had a personal reason for choosing the project	3.1	0.9				D
Emotions						
Delighted	3.7	0.8	A			
Happy	3.7	1.1	A			
Joyful	3.4	1.0	A			
Amazed	3.0	1.0		B		
Surprised	2.8	0.9		B		
Astonished	2.7	0.9		B		

Items are grouped into two categories: project ownership (five-point Likert scale: 1 = strongly disagree, 5 = strongly agree) and emotions associated with project experience (five-point Likert-like scale: 1 = very slightly, 5 = very strongly). Average (Avg) and standard deviation (SD) are reported, and items are ordered by average from highest to lowest value within each category. Columns A-D: Items within each column are not statistically different, whereas items in different columns are statistically different, based on separate ANOVAs followed by Tukey's HSD tests, one for ownership and one for emotions.

TABLE 5 | Classroom community inventory.

Item	Avg	SD
Peer support	3.4	0.7
I trust others in this course	3.5	0.8
I feel that students in this course care about each other	3.4	0.8
I feel connected to others in this course	3.4	0.9
I feel confident that others in this course will support me	3.4	0.8
I feel that I can rely on others in this course	3.4	0.9
Learning support	3.1	0.4
I feel that I am given ample opportunities to learn in this course	3.7	0.8
I feel that I receive timely feedback in this course	3.6	0.8
* I feel that this course results in only modest learning	3.3	0.8
* I feel that my educational needs are not being met in this course	2.7	1.0
* I feel that this course does not promote a desire to learn	2.4	1.0

*Items are grouped into two dimensions of peer support and learning support. Each dimension consists of five related items on a five-point Likert scale (1 = strongly disagree, 5 = strongly agree). Average (Avg) and standard deviation (SD) for each dimension and item are reported. Items with * are reverse coded, and ratings are reported after being converted to the positive scale.*

support includes items such as "I feel connected to others in this course" and "I feel that I can rely on others in this course." Learning support includes items such as "I feel that I am given ample opportunities to learn in this course" and "I feel that my educational needs are not being met in this course" (reverse-coded item). These results are similar to those in the original literature, with ratings in peer support and learning support at 3.3 ± 0.5 and 2.9 ± 0.9, respectively (Rovai et al., 2004).

The laboratory course assessment survey provides additional information on how students perceived the presence of three of the five CURE design elements: iteration, discovery, and collaboration (**Table 6**). Students reported average post-course ratings of 4.2 ± 0.8 and 4.1 ± 0.8 (on a six-point Likert-like scale) for the iteration and discovery dimensions, respectively. The iteration dimension includes items such as "share and compare data with other students," and the discovery dimension includes items such as "develop new arguments based on data." In the course, students completed 3–4 writing assignments, in which they constructed scientific arguments to draw conclusions based on data in the laboratory. These writing assignments asked students to use data collected and analyzed by all student teams in the course. In laboratory sections, student teams posted their data on Google Spreadsheet files to facilitate the sharing of data. Student teams were also asked compare their own data with those from other teams as they data were shared.

For collaboration, which include items such as "discuss elements of my investigation with classmates or instructors" and "help other students collect or analyze data," students reported a post-course rating of 3.5 ± 0.6 (on a four-point frequency scale: 1 = never, 2 = one or two times, 3 = monthly, and 4 = weekly). Laboratory sections met once for 3 h each week, and students always worked in teams to collect and analyze data. Student teams also developed their research proposals in a scaffolded fashion with dedicated work time in laboratory sections and milestones throughout the quarter. Therefore, it was likely that these items in the collaboration dimension have happened weekly or almost weekly.

TABLE 6 | Laboratory course assessment survey.

Item	Avg	SD
Iteration	4.2	0.8
Share and compare data with other students	4.7	0.8
Revise drafts of papers or presentations based on feedback	4.3	1.1
Revise or repeat analyses based on feedback	4.1	1.1
Collect and analyze additional data to address new questions	3.9	1.2
Change the methods of the investigation	3.8	1.2
Discovery	4.1	0.8
Formulate my own research questions or hypothesis	4.3	1.0
Develop new arguments based on data	4.2	1.0
Revise or repeat work to account for errors or fix problems	4.2	1.1
Explain how my work has resulted in new scientific knowledge	4.2	1.1
Conduct an investigation to find something previously unknown	4.1	1.0
Generate novel results that could be of interest to the community	3.6	1.2
Collaboration	3.5	0.6
Discuss elements of my investigation with classmates or instructors	3.7	0.6
Reflect on what I was learning with others	3.7	0.7
Share problems and seek input on how to address them	3.6	0.8
Contribute my ideas and suggestions during class discussions	3.5	0.8
Help other students collect or analyze data	3.4	0.9
Provide constructive criticism and challenge each other's interpretations	3.1	1.0

Items are grouped into three dimensions related to some of the CURE design elements: iteration, discovery, and collaboration. Items in the iteration and discovery dimensions are on a six-point Likert-like scale (1 = strongly disagree, 6 = strongly agree), and items in the collaboration dimension are on a four-time frequency scale (1 = never, 4 = weekly). Average (Avg) and standard deviation (SD) for each dimension and item are reported.

DISCUSSION

In this paper, we report student outcomes from a CURE on soil microbiomes situated in a large-enrollment introductory biology laboratory course. Early research experiences are critical to student learning, as well as identity formation and persistence in STEM, and CUREs in introductory courses can play an important role in promoting student success (President's Council of Advisors on Science, and Technology [PCAST], 2012). Compared to many other examples in the existing literature, this CURE is unique in two ways. The course is required for all biological sciences majors at the study institution and does not have any prerequisites, thus providing universal access to research experiences for all beginning undergraduate students before they are likely to encounter the negative weed-out environment common in introductory STEM courses (Mervis, 2011).

Learning activities in this soil microbiome course were intentionally developed based on the five CURE design elements (**Table 1**). The intended curriculum (designed by educators based on learning principles) can be substantially different from what students experience in the classroom (Bussey et al., 2013; Lloyd et al., 2017). Therefore, it is important to examine the student perspectives. In post-course surveys, students reported ratings in agreement with the presence of the CURE design elements. Three of the five design elements (collaboration, iteration, and discovery) were observed in the laboratory course assessment survey (**Table 6**). Certain items in the project ownership survey, including "my research will help to solve a problem in the world" and "my findings were important to the scientific community," directly relate to relevance, and students reported agreement with the presence of this design element (**Table 5**). For scientific practices, students reported significant gains in self-efficacy on research skills, with effect sizes larger than gains in self-efficacy on general course skills (**Table 4**). In the category of experiences with different types of projects, students reported moderate effect sizes in the items related to doing a project where "no one knows the outcome," "students have some input," and "entirely designed by students" but only small effect sizes for projects where "instructor knows the outcomes," "structured by the instructor," and "students know the outcome" (**Table 4**), further suggesting the presence of the scientific practices CURE design element.

Student outcomes in this paper are primarily observed through pre- and post-course surveys on self-efficacy on research skills, and students reported significant gains in 22 out of 25 items from the classroom undergraduate research experience survey (**Table 3**). In parallel, within the course, students completed writing assignments and poster presentations that were graded as summative assessments to determine if they have achieved the course learning objectives, even though these artifacts were not included as part of this study. Furthermore, while self-efficacy is not the same as cognitive performance on assessment tasks, affective considerations are important for student persistence in STEM. In fact, students from minoritized backgrounds leave STEM majors at disproportionately higher rates compared to students from majority and dominant cultures, and this exclusion is not primarily related to academic performance (Seymour and Hewitt, 1997; Asai, 2020). Compared to two decades ago, a higher percentage of students today report negative teaching and learning experiences related to the affective domain as reasons for leaving STEM majors (Seymour and Hewitt, 1997; Seymour and Hunter, 2019). Self-efficacy is a key affective component in science identity (Carlone and Johnson, 2007; Hazari et al., 2013), and research experiences can help promote the development of science identity through increasing self-efficacy (Graham et al., 2013). Therefore, it is reasonable to expect that increased self-efficacy from CUREs such as the one described here will ultimately lead to higher persistence in STEM.

DATA AVAILABILITY STATEMENT

Data are available upon reasonable request and with permission of the Institutional Review Board.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of California, San Diego, Human Research Protections Program. Written informed consent for

participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SL designed the study, collected and analyzed data, and wrote the manuscript. BL analyzed data and generated tables. Both authors contributed to the article and approved the submitted version.

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REFERENCES

American Association for the Advancement of Science [AAAS] (1989). *Science for All Americans*. Oxford: Oxford University Press.

Armstrong, R. L. (1987). The midpoint on a five-point Likert-type scale. *Percept. Mot. Skills* 64, 359–362. doi: 10.2466/pms.1987.64.2.359

Asai, D. J. (2020). Race matters. *Cell* 181, 754–757.

Auchincloss, L. C., Laursen, S. L., Branchaw, J. L., Eagan, K., Graham, M., Hanauer, D. I., et al. (2014). Assessment of course-based undergraduate research experiences: a meeting report. *CBE Life Sci. Educ.* 13, 29–40. doi: 10.1187/cbe.14-01-0004

Bangera, G., and Brownell, S. E. (2014). Course-based undergraduate research experiences can make scientific research more inclusive. *CBE Life Sci. Educ.* 13, 602–606. doi: 10.1187/cbe.14-06-0099

Ben-Nun, P. (2008). "Respondent fatigue," in *Encyclopedia of Survey Research Methods*, ed. P. J. Lavrakas (Thousand Oaks, CA: Sage Publications).

Bhatt, J. M., and Challa, A. K. (2018). First year course-based undergraduate research experience (CURE) using the CRISPR/Cas9 genome engineering technology in zebrafish. *J. Microbiol. Biol. Educ.* 19:19.1.3.

Boyer Commission (1998). *Reinventing Undergraduate Education: a Blueprint for America's Research Universities*. New York: Stoney Brook University.

Brownell, S. E., and Kloser, M. J. (2015). Toward a conceptual framework for measuring the effectiveness of course-based undergraduate research experiences in undergraduate biology. *Stud. Higher Educ.* 40, 525–544. doi: 10.1080/03075079.2015.1004234

Buck, L. B., Bretz, S. L., and Towns, M. H. (2008). Characterizing the level of inquiry in the undergraduate laboratory. *J. Coll. Sci. Teach.* 38, 52–58.

Bussey, T. J., Orgill, M., and Crippen, K. J. (2013). Variation theory: a theory of learning and a useful theoretical framework for chemical education research. *Chem. Educ. Res. Pract.* 14, 9–22. doi: 10.1039/c2rp20145c

Butler, M., Henter, H., and Mel, S. (2014). From bugs to barcodes: using molecular tools to study biodiversity. *Tested Stud. Laboratory Teach.* 35, 41–55.

Carlone, H. B., and Johnson, A. (2007). Understanding the science experiences of successful women of color: science identity as an analytic lens. *J. Res. Sci. Teach.* 44, 1187–1218. doi: 10.1002/tea.20237

Caspers, M. L., and Roberts-Kirchhoff, E. S. (2003). An undergraduate biochemistry laboratory course with an emphasis on a research experience. *Biochem. Mol. Biol. Educ.* 31, 303–307. doi: 10.1002/bmb.2003.494031050257

Chen, J., Call, G. B., Beyer, E., Bui, C., Cespedes, A., and Chan, A. (2005). Discovery-based science education: functional genomic dissection in Drosophila by undergraduate researchers. *PLoS Biol.* 3:e59.

Corwin, L. A., Graham, M. J., and Dolan, E. L. (2015a). Modeling course-based undergraduate research experiences: an agenda for future research and evaluation. *CBE Life Sci. Educ.* 14:es1.

Corwin, L. A., Runyon, C., Robinson, A., and Dolan, E. L. (2015b). The laboratory course assessment survey: a tool to measure three dimensions of research-course design. *CBE Life Sci. Educ.* 14:ar37. doi: 10.1187/cbe.15-03-0073

Davis, E., Sloan, T., Aurelius, K., Barbour, A., Bodey, E., Clark, B., et al. (2017). Antibiotic discovery throughout the small World initiative: a molecular strategy to identify biosynthetic gene clusters involved in antagonistic activity. *MicrobiologyOpen* 6:e00435. doi: 10.1002/mbo3.435

Denofrio, L. A., Russell, B., Lopatto, D., and Lu, Y. (2007). Linking student interests to science curricula. *Science* 318, 1872–1873. doi: 10.1126/science.1150788

Desai, K. V., Gatson, S. N., Stiles, T. W., Stewart, R. H., Laine, G. A., and Quick, C. M. (2008). Integrating research and education at research-extensive universities with research-intensive communities. *Adv. Physiol. Educ.* 32, 136–141. doi: 10.1152/advan.90112.2008

Edwards, T. M., Smith, B. K., Watts, D. L., Germain-Aubrey, C. C., Roark, A. M., Bybee, S. M., et al. (2011). Group-advantaged training of research (GATOR): a metamorphosis of mentorship. *BioScience* 61, 301–311. doi: 10.1525/bio.2011.61.4.10

Graham, M. J., Frederick, J., Byars-Winston, A., Hunter, A. B., and Handelsman, J. (2013). Increasing persistence of college students in STEM. *Science* 341, 1455–1456. doi: 10.1126/science.1240487

Guy, R. F., and Norvell, M. (1977). The neutral point on a likert scale. *J. Psychol.* 95, 199–204. doi: 10.1080/00223980.1977.9915880

Hanauer, D. I., and Dolan, E. L. (2014). The project ownership survey: measuring differences in scientific inquiry experiences. *CBE Life Sci. Educ.* 13, 149–158. doi: 10.1187/cbe.13-06-0123

Hanauer, D. I., Jacobs-Sera, D., Pedulla, M. L., Cresawn, S. G., Hendrix, R. W., and Hatfull, G. F. (2006). Teaching scientific inquiry. *Science* 314, 1880–1881.

Hazari, Z., Sadler, P. M., and Sonnert, G. (2013). The science identity of college students: exploring the intersection of gender, race, and ethnicity. *J. Coll. Sci. Teach.* 42, 82–91.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* 6, 65–70.

Horowitz, J., and Christopher, K. B. (2013). The research mentoring program: serving the needs of graduate and undergraduate researchers. *Innov. High. Educ.* 38, 105–116. doi: 10.1007/s10755-012-9230-3

Hunter, A.-B., Laursen, L. S., and Seymour, E. (2006). Becoming a scientist: the role of undergraduate research in students' cognitive, personal, and professional development. *Sci. Educ.* 91, 36–74. doi: 10.1002/sce.20173

Kinkel, D. H., and Henke, S. E. (2006). Impact of undergraduate research on academic performance, educational planning, and career development. *J. Nat. Resour. Life Sci. Educ.* 35, 194–201. doi: 10.2134/jnrlse2006.0194

Kloser, M. J., Brownell, S. E., Chiariello, N. R., and Fukami, T. (2011). Integrating teaching and research in undergraduate biology laboratory education. *PLoS Biol.* 9:e1001174. doi: 10.1371/journal.pbio.1001174

Kloser, M. J., Brownell, S. E., Shavelson, R. J., and Fukami, T. (2013). Effects of a research-based ecology lab course: a study of nonvolunteer achievement, self-confidence, and perception of lab course purpose. *J. Coll. Sci. Teach.* 42, 72–81.

Komorita, S. S. (1963). Attitude content, intensity, and the neutral point on a Likert scale. *J. Soc. Psychol.* 61, 327–334. doi: 10.1080/00224545.1963.9919489

Kuh, G. D. (2008). *High-Impact Educational Practices: What They Are, Who Has Access to Them, and Why They Matter*. Washington, DC: The Association of American Colleges and Universities.

Lave, J., and Wenger, E. (1991). *Situated Learning: Legitimate Peripheral Participation*. New York, NY: Cambridge University Press.

Light, C. J., Fegley, M., and Stamp, N. (2019). Emphasizing iterative practices for a sequential course-based undergraduate research experience in microbial biofilms. *FEMS Microbiol. Lett.* 366:fnaa001.

Lloyd, G. M., Cai, J., and Tarr, J. E. (2017). "Issues in curriculum studies: evidence-based insights and future directions," in *Compendium for Research in Mathematics Education*, ed. J. Cai (Reston, VA: The National Council of Teachers of Mathematics), 824–852.

Lo, S. M., and Mel, S. F. (2017). Examining microbial biodiversity in soil: a large-enrollment introductory course-based undergraduate research experience. *Tested Stud. Laboratory Educ.* 38:6.

Lo, S. M., and Mordacq, J. C. (2020). "Course-based undergraduate research experiences in biology," in *Active Learning College Science: the Case for Evidence Based Practice*, eds J. J. Mintzes and E. M. Walter. (Berlin: Springer), 467–479.

Lopatto, D., Alvarez, C., Barnard, D., Chandrasekaran, C., Chung, H. M., and Du, C. (2008). Genomics education partnership. *Science* 322, 684–685.

Maher, J. M., Markey, J. C., and Ebert-May, D. (2013). The other half of the story: effect size analysis in quantitative research. *CBE Life Sci. Educ.* 12, 345–351. doi: 10.1187/cbe.13-04-0082

McCormick, A. C., and Zhao, C. M. (2005). Rethinking and reframing the Carnegie classification. *Change Magazine High. Learn.* 37, 51–57. doi: 10.3200/chng.37.5.51-57

Mervis, J. (2011). Weed-out courses hamper diversity. *Science* 334:1333. doi: 10.1126/science.334.6061.1333

Mordacq, J. M., Drane, D. L., Swarat, S. L., and Lo, S. M. (2017). Development of course-based undergraduate research experiences using a design-based approach. *J. Coll. Sci. Teach.* 46, 64–75.

Murthy, P. P. N., Thompson, M., and Hungwe, K. (2014). Development of a semester-long, inquiry-based laboratory course in upper-level biochemistry and molecular biology. *J. Chem. Educ.* 91, 1909–1917. doi: 10.1021/ed400119u

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858. doi: 10.1038/35002501

National Academies of Sciences, Engineering, and Medicine [NASEM] (2016). *Barriers, and Opportunities for 2-Year and 4-Year STEM Degrees: Systemic Change to Support Students' Diverse Pathways*. Washington, DC: The National Academies Press.

National Academy of Sciences [NAS], National Academy of Engineering, and Institute of Medicine. (2011). *Expanding Underrepresented Minority Participation: America's Science and Technology Talent at the Crossroads*. Washington, DC: The National Academies Press.

National Research Council [NRC] (2003). *BIO2010: Transforming Undergraduate Education for Future Research Biologists*. Washington, DC: National Academies Press.

Palmer, R. J., Hunt, A. N., Neal, M., and Wuetherick, B. (2015). Mentoring, undergraduate research, and identity development: a conceptual review and research agenda. *Mentor. Tutoring Partnership Learn.* 23, 411–426. doi: 10.1080/13611267.2015.1126165

Pedwell, R. K., Fraser, J. A., Wang, J. T., Clegg, J. K., Chartres, J. D., and Rowland, S. L. (2018). The beer and biofuels laboratory: a report on implementing and supporting a large, interdisciplinary, yeast-focused course-based undergraduate research experience. *Biochem. Mol. Biol. Educ.* 46, 213–222. doi: 10.1002/bmb.21111

President's Council of Advisors on Science, and Technology [PCAST] (2012). *Engage to Excel: Producing One Million Additional College Graduates with Degrees in Science, Technology, Engineering, and Mathematics*. Washington, DC: PCAST.

Rodenbusch, S. E., Hernandez, P. R., Simmons, S. L., and Dolan, E. L. (2016). Early engagement in course-based research increases graduation rates and completion of science, engineering, and mathematics degrees. *CBE Life Sci. Educ.* 15:ar20. doi: 10.1187/cbe.16-03-0117

Rovai, A. P., Wighting, M. J., and Lucking, R. (2004). The classroom and school community inventory: development, refinement, and validation of a self-report measure for educational research. *Internet High. Educ.* 7, 263–280. doi: 10.1016/j.iheduc.2004.09.001

Seymour, E., and Hewitt, N. M. (1997). *Talking About Leaving: Why Undergraduates Leave the Sciences*. Boulder, CO: Westview Press.

Seymour, E., and Hunter, A. B. (2019). *Talking about Leaving Revisited: Persistence, Relocation, and Loss in Undergraduate STEM Education*. New York, NY: Springer.

Shaffer, J. P. (1995). Multiple hypothesis resting. *Annu. Rev. Psychol.* 46, 561–584.

Shapiro, C., Moberg-Parker, J., Toma, S., Ayon, C., Zimmerman, H., Roth-Johnson, E. A., et al. (2015). Comparing the impact of course-based and apprentice-based research experiences in a life science laboratory curriculum. *J. Microbiol. Biol. Educ.* 16, 186–197. doi: 10.1128/jmbe.v16i2.1045

Summers, M. F. (2011). Training the next generation of protein scientists. *Protein Sci.* 20, 1796–1801. doi: 10.1002/pro.733

Summers, M. F., and Hrabowski, F. A. (2006). Preparing minority scientists and engineers. *Science* 311, 1870–1871. doi: 10.1126/science.1125257

Taylor, E. V., Fortune, J. A., and Drennan, C. L. (2010). A research-inspired laboratory sequence investigating acquired drug resistance. *Biochem. Mol. Biol. Educ.* 38, 247–252. doi: 10.1002/bmb.20384

Thompson, S. K., Neill, C. J., Wiederhoeft, E., and Cotner, S. (2016). A model for a course-based undergraduate research experience (CURE) in a field setting. *Jo. Microbiol. Biol. Educ.* 17, 469–471. doi: 10.1128/jmbe.v17i3.1142

Weaver, G. C., Russell, C. B., and Wink, D. J. (2008). Inquiry-based and research-based laboratory pedagogies in undergraduate science. *Nat. Chem. Biol.* 4, 577–580. doi: 10.1038/nchembio1008-577

Wenger, E. (1999). *Communities of Practice: Learning, Meaning, and Identity*. New York, NY: Cambridge University Press.

Wilcoxon, F. (1945). Individual comparisons by ranking methods. *Biometrics Bull.* 1, 80–83. doi: 10.2307/3001968

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