frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 08 January 2021
doi: 10.3389/fevo.2020.614367

Metabolic Symbiosis Facilitates Species Coexistence and Generates Light-Dependent Priority Effects

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Metabolic symbiosis is a form of symbiosis in which organisms exchange metabolites, typically for mutual benefit. For example, acquired phototrophs like *Paramecium bursaria* obtain photosynthate from endosymbiotic green algae called *Chlorella.* In addition to facilitating the persistence of *P. bursaria* by providing a carbon source that supplements *P. bursaria*'s heterotrophic digestion of bacteria, symbiotic *Chlorella* may impact competitive interactions between *P. bursaria* and other bacterivores, with cascading effects on community composition and overall diversity. Here, we tested the effects of metabolic symbiosis on coexistence by assessing the impacts of acquired phototrophy on priority effects, or the effect of species arrival order on species interactions, between *P. bursaria* and its competitor *Colpidium*. Our results suggest light-dependent priority effects. The acquired phototroph benefited from metabolic symbiosis during sequential arrival of each organism in competition, and led to increased growth of late-arriving *Colpidium*. These findings demonstrate that understanding the consequences of priority effects for species coexistence requires consideration of metabolic symbiosis.

Keywords: acquired photosynthesis, ciliates, *Colpidium*, *Paramecium bursaria*, protist microcosm

Edited by:
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Specialty section:
This article was submitted to
Coevolution,
a section of the journal
Frontiers in Ecology and Evolution

Received: *05 October 2020*
Accepted: *09 December 2020*
Published: *08 January 2021*

Citation:
Hsu V and Moeller HV (2021)
Metabolic Symbiosis Facilitates
Species Coexistence and Generates
Light-Dependent Priority Effects.
Front. Ecol. Evol. 8:614367.
doi: 10.3389/fevo.2020.614367

INTRODUCTION

Metabolic symbiosis is a form of symbiosis in which organisms exchange metabolites, typically for mutual benefit. In some cases, metabolic partners are capable of integrating metabolic pathways across genomes to produce shared metabolites, such as amino acids and vitamins, for mutual survival (Husnik et al., 2013), and other organisms can directly use metabolites produced by a partner organism for energy or nutrient sources (Estrela et al., 2012). Symbiotic mycorrhizal fungi benefit plant hosts by increasing the efficiency of nutrient acquisition in exchange for carbohydrates (Marschner and Dell, 1994). Similarly, planktonic ciliates, such as *Paramecium bursaria,* host algae in exchange for photosynthates (Stoecker et al., 2009; Iwai et al., 2019). Symbiotic relationships, such as mutualisms that benefit the two organisms involved in the symbiosis, can also have important implications for community dynamics by promoting diversity, population stability (Pachepsky et al., 2002; Mougi and Kondoh, 2012), and even co-evolution (Guimarães et al., 2011). Metabolic symbiosis is context-dependent: environmental conditions are known to shift mutually beneficial relationships into host-parasitism when costs begin to outweigh benefits of symbiosis (Bronstein, 1994; Johnson et al., 1997; Lowe et al., 2016).

When organisms receive metabolic benefits from a symbiont, this symbiosis can increase competitive ability and facilitate coexistence between competitors. In plant and mycorrhizal symbiosis, fungal partners may increase competitive abilities of host plant species through differential benefits (Callaway et al., 2001), and may also mediate competition through common mycorrhizal networks within a plant population (Francis and Read, 1984; Grime et al., 1987). Acquired phototrophs, such as *P. bursaria*, can also become a better competitor by gaining access to a new resource via acquisition of photosynthetic machinery through their endosymbionts (Moeller et al., 2016). However, the extent of these benefits can vary. The growth rate of *P. bursaria* with endosymbiotic algae depends on temperature (Salsbery and DeLong, 2018) and light level (Pado, 1965; Weis, 1974). Here, we study the role of metabolic symbiosis on species coexistence under different community assemblage scenarios.

The effects of metabolic symbiosis on competitive outcomes may also be impacted by the particular timing and order in which species join a community, a phenomenon known as priority effects (Chase, 2003; Fukami, 2015). In other words, the competitive ability of a species can change based on its arrival order relative to its competitor. Priority effects have been studied across taxonomic domains between yeast and bacteria (Fukami, 2015), between functional groups in plants (Weidlich et al., 2018), and under various environmental conditions (Vannette and Fukami, 2014). The outcomes of priority effects are highly variable across species and ecosystems (Fukami, 2015). When priority effects are present, there are two possible outcomes: facilitation, in which the early arriving species positively influences the growth of the late-arriving species, or inhibition, where the opposite is true (Fukami, 2015). The impact of a metabolic symbiosis on the strength of priority effects has not been extensively investigated.

In this study, we used two bacterivorous ciliate competitors, *Paramecium bursaria*, and *Colpidium* sp., to test for priority effects. *Colpidium* is a strict heterotroph while *P. bursaria* engages in metabolic symbiosis with its algal endosymbiont, *Chlorella*. *Paramecium bursaria* almost always host *Chlorella*, yet is known to harbor endosymbionts that come from at least five different species, including *Coccomyxa simplex* and *Micractinium conductrix* (Hoshina and Imamura, 2008; Johnson, 2011; Pröschold et al., 2011). In the presence of light, endosymbiotic *Chlorella* undergoes photosynthesis and provides sugar metabolites for *P. bursaria* presumably in exchange for protection (Karakashian, 1963; Pado, 1965; Weis, 1974). *Paramecium bursaria* also provides essential nitrogen by prey cell digestion for *Chlorella's* nutritional requirement, and *P. bursaria* cells with endosymbionts have increased bacterial ingestion (Albers and Wiessner, 1985; Johnson, 2011). To understand the role of metabolic symbiosis on species coexistence, we tested the strength of priority effects between *P. bursaria* and *Colpidium*. We manipulated light level to control the contribution of photosynthesis on *P. bursaria*'s growth. We hypothesized that as light level increased, *Chlorella* would also increase its photosynthetic capacity, thereby providing more metabolites for *P. bursaria*'s growth. In darkness, hosting photosynthetic endosymbionts becomes too costly and confers no energetic benefits, so *Chlorella* would likely be expelled and *P. bursaria* would rely entirely on heterotrophy (Lowe et al., 2016). In our study, we did not observe bleached *P. bursaria*, suggesting that expulsion, if it was occurring, proceeded at slower timescales than death of *P. bursaria*. The two species were inoculated sequentially to mimic differential species assemblage, and the strength of priority effects were measured by comparing species densities across treatments. Our results suggested light-dependent priority effects. The acquired phototrophs persisted in the presence of metabolic symbiosis with endosymbiotic algae when the competitors assembled sequentially, and even facilitated the growth of late-arriving *Colpidium*. Our findings demonstrated that understanding the consequences of priority effects for species coexistence and overall maintenance of biodiversity requires consideration of metabolic symbiosis.

METHODOLOGY

Cultures of *Colpidium* sp., *P. bursaria*, and bacterial species (see below) were obtained from Carolina Biological Supply company. To allow *Colpidium* and *P. bursaria* to adjust to the light environments corresponding to the experimental conditions, the laboratory stock cultures were maintained at 50, 100, and 200 μmol quanta m^{-2} s^{-1} in an incubator at 24°C under controlled light conditions (12 h light: 12 h dark) for at least 2 weeks prior to experimentation.

Initially sterilized protozoan pellet media (Carolina Biological Supply Company) was inoculated with bacteria 2 days prior to the start of experimentation to control for bacterial prey availability in experimental microcosms. We inoculated treatments with three species of bacteria: (1) *Serratia marcescens* and the dominant bacterial species from purchased protist cultures of (2) *P. bursaria* and (3) *Colpidium*. The purchased protist cultures were plated onto Nutrient Agar (Research Products International), and we isolated the most abundant bacterial morphotype to identify the dominant bacterial morphospecies co-occurring with *P. bursaria* and *Colpidium*. These bacterial morphospecies were transferred from the agar plates to liquid media culture to be inoculated into treatment microcosms. Bacterizing experimental media with the same three bacterial morphospecies ensured that each treatment culture was primed with the same key bacterial prey community members.

To test how photosynthetic symbiosis impacted the strength of priority effects, we sequentially inoculated *P. bursaria* and *Colpidium* into experimental microcosms at four different light levels (0, 50, 100, 200 μmol quanta m^{-2} s^{-1}) in triplicate. The two introduction treatments were: (i) "*P. bursaria*-first" sequential inoculations, in which *P. bursaria* was introduced first on Day 0, and *Colpidium* on Day 14; (ii) "*Colpidium*-first," in which *Colpidium* was introduced first on Day 0, and *P. bursaria* on Day 7. The intrinsic growth rate and carrying capacity of both species were determined by conducting single-ciliate control treatments. In single-species treatments, *Colpidium* had a much higher intrinsic growth rate, and stopped growing exponentially by Day 7, while *P. bursaria* required 14 days for its populations to stabilize. Because, in our priority effects experiments, we wanted *P. bursaria* and *Colpidium* to grow to their maximum population sizes before the introduction of the second species, we decided to allow *P. bursaria* 14 days of growth before

Colpidium inoculation for *P. bursaria*-first treatments, and 7 days for *Colpidium* in *Colpidium*-first treatments. Thus the total experiment contained 48 microcosms: 2 priority effect treatments (*P. bursaria*-first, *Colpidium*-first) × 4 light levels (0, 50, 100, 200 μmol quanta m^{-2} s^{-1}) × 3 replicates, plus 2 single-species controls (*P. bursaria* only, *Colpidium* only) × 4 light levels × 3 replicates.

At the start of each experiment, we inoculated 50 ciliates/mL into the experimental microcosms with 40 mL of liquid media. For priority effects treatments, we subsequently inoculated 50 ciliates/mL of the second species on Day 7 (*Colpidium*-first) or Day 14 (*P. bursaria*-first) of the experiment. We quantified population densities of each ciliate three times per week over the duration of the experiment (40 days). During sampling, an aliquot containing at least 50 ciliate cells (50–1,000 μL) was removed from each microcosm, and the ciliates were individually counted using a dissecting microscope. We used a range of aliquot sample volumes because the population sizes throughout the duration of the experiment (40 days) often differed up to two orders of magnitude (**Supplementary Figure S1**). More specifically, during the first week, we removed 1 mL from each experimental flask for enumeration, during the second and third weeks we removed 200 μL, and during the subsequent weeks we removed 50 μL.

We quantified a priority effects metric (modified from Vannette and Fukami, 2014) by comparing the maximum population sizes achieved by each species when introduced first [$D(i)_{ij}$] to maximum population when introduced last [$D(i)_{ji}$]:

$$P_{ij} = \ln\left[D\left(i\right)_{ji} / D\left(i\right)_{ij}\right] \quad (1)$$

The order of the subscripts *i* and *j* indicate arrival order of the species. By taking the natural log of this ratio of population sizes, we achieve a metric for which positive values indicate positive priority effects (e.g., lower population sizes when introduced first, indicating that the early presence of the other species benefited the later arriving species) and negative values indicate negative priority effects (e.g., lower population sizes when introduced last, indicating that the early presence of the other species inhibited growth of the later arriving species).

To confirm that observed priority effects were not due to the presence of free-living *Chlorella* cells, we visually inspected stock cultures and experimental cultures using light microscopy for the presence of *Chlorella* colonies. We also performed chlorophyll-*a* extractions on unspent media (media that had never been inoculated with *P. bursaria*) as well as spent media from experimental cultures at all light levels. We obtained this spent media by passaging 1 mL of culture volume through an 8.0 mm transwell membrane filter (Corning Incorporated, Corning, NY, United States), and confirming using light microscopy that the filtrate did not contain any *P. bursaria* cells. We then collected this filtrate on a GF/F filter (Whatman, SigmaAldrich, St. Louis, MO, United States), placed the filter in 5 mL of a 90% acetone solution in water, and incubated overnight at -20°C. The following day, we measured chlorophyll-*a* content in relative fluorescence units using a Trilogy fluorometer with a 450 nm LED (Turner Designs, San Jose, CA).

We performed all data analyses and plotting using the open-source software package R (R Core Team, 2020). Our analysis script and data files can be found at https://doi.org/10.5281/zenodo.4342275.

RESULTS

Paramecium bursaria was negatively affected by early arriving *Colpidium*, with *P. bursaria* populations visibly lower in all *Colpidium*-first treatments and nearly competitively excluded at the end of 40 days (**Figures 1A–D**). Conversely, *Colpidium* grew to higher population sizes when *P. bursaria* was introduced first, and density differences between *Colpidium*-first and *P. bursaria*-first treatments at each light level increased with increasing irradiance (**Figures 1E–H**). The calculated strength of priority effects using the metric defined by Eq. 1 supported these observations (**Figure 2** and **Supplementary Table S1**). *P. bursaria* experienced negative priority effects, which indicates that early arriving *Colpidium* inhibited the growth of *P. bursaria* (**Figure 2A**). **Figure 2B** shows the strength of priority effects by light level, and at 0 μmol quanta m^{-2} s^{-1}, *P. bursaria* populations died off independent of *Colpidium* (**Figures 1**, **2B**). Yet increasing light level (from 50 to 200 μmol quanta m^{-2} s^{-1}) did not enhance the negative affect of *Colpidium* on *P. bursaria* (**Figure 2B**). Conversely, *P. bursaria* had a positive priority effect on *Colpidium* (**Figure 2A**), and this positive priority effect strengthened with increasing light (**Figure 2B**). *Colpidium* populations at 100 and 200 μmol quanta m^{-2} s^{-1} both benefited from pre-established *P. bursaria*, with significantly higher positive priority effects at 200 μmol quanta m^{-2} s^{-1} (**Figure 2B**).

We obtained maximum population sizes within the 40-day duration of the experiment (**Figures 3A,B**) and growth rates captured the initial exponential growth period before reaching carrying capacity, calculated from the first 10 days for *Colpidium*, and 20 days for *P. bursaria* (**Figures 3C,D**). Comparison of priority effect treatments with single-species controls demonstrated that presence of any competitor, regardless of arrival order, inhibited *P. bursaria* growth (**Figure 1**). In high light (100 and 200 μmol quanta m^{-2} s^{-1}), *P. bursaria* maximum population abundances were significantly lower in the presence of *Colpidium* competitors (ANOVA $F = 47.14$, $p < 0.01$, **Supplementary Table S2**), and were visibly lower when *Colpidium* was introduced first (**Figure 3A**). Even when *P. bursaria* had pre-established populations before *Colpidium* was introduced in *P. bursaria*-first treatments, the later introduction of *Colpidium* resulted in lower maximum population sizes of *P. bursaria* (**Figure 3A**). The initial growth rates were not significantly different between the *P. bursaria* controls and *P. bursaria*-first treatments (**Figure 3C**), so lower populations sizes were not explained by initial growth rates. When *P. bursaria* was introduced second, however, the priority effect was strong enough to inhibit initial growth rates (**Figure 3C**) and ultimately, population sizes (**Figure 3A**).

Colpidium population densities were generally greater in the presence of *P. bursaria,* especially when *P. bursaria* arrived first

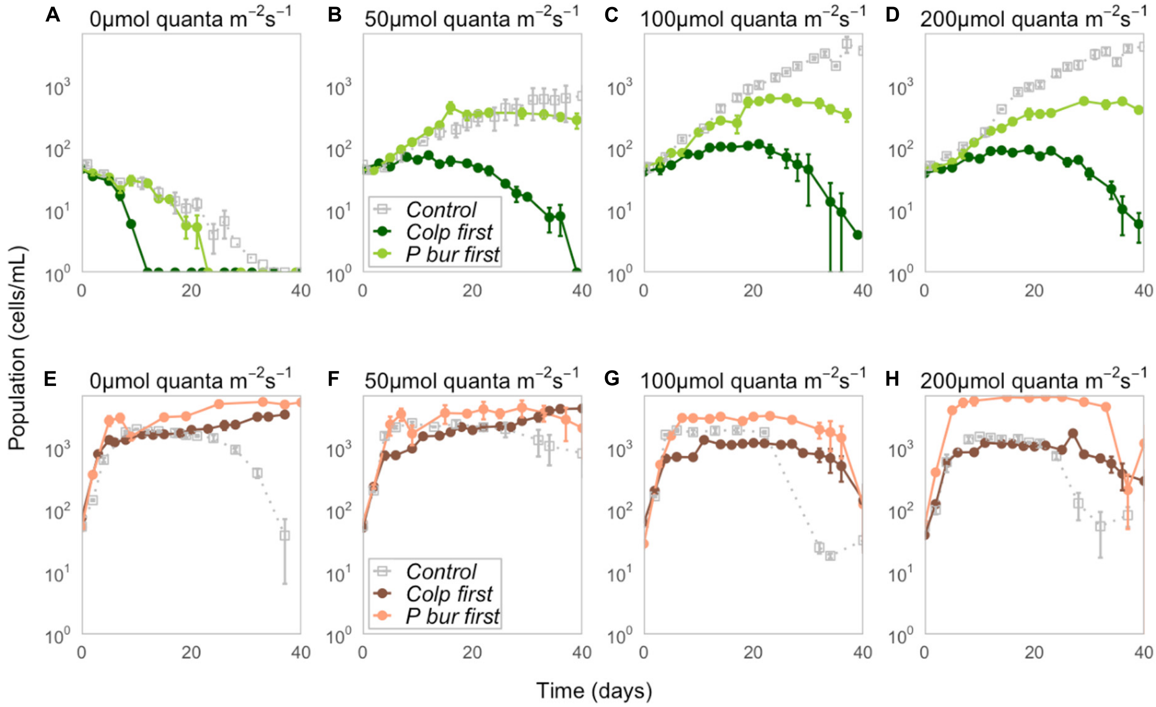

FIGURE 1 | Top row **(A–D)** Population dynamics of *P. bursaria* for control (gray), *Colpidium*-first (dark green), and *P. bursaria*-first (light green) treatments at four different light levels (0, 50, 100, and 200 μmol quanta m^{-2} s^{-1}). Time is measured in days since inoculation, rather than experimental day, so that $t = 0$ represents the date on which the focal (plotted) species was inoculated. (see **Supplementary Figure S1** for population size as a function of experimental day). Bottom row **(E–H)** Population dynamics of *Colpidium* for control (gray), *Colpidium*-first (dark coral), and *P. bursaria*-first (light coral) treatments at four different light levels (0, 50, 100, and 200 μmol quanta m^{-2} s^{-1}). Note that population densities were recorded on a logarithmic scale, and error bars represent ± one standard error.

(**Figure 3B**). However, there was an exception in *Colpidium*-first treatments at 100 and 200 μmol quanta m^{-2} s^{-1}, in which early arriving *Colpidium* did not benefit from the subsequent introduction of *P. bursaria* (**Figure 3B**). Effects on *Colpidium* were generally not mediated by initial growth rate, suggesting alleviation of later resource limitation was responsible for priority effects (**Figure 3D**). We found no evidence of free-living *Chlorella* cells in either microscope assays or through increases in chlorophyll-*a* fluorescence (**Supplementary Figure S2**).

DISCUSSION

Our results highlight the importance of acquired metabolism to community assembly. In our study, *P. bursaria*, whose photosynthetic symbionts provide a secondary source of energy, facilitated the later arrival of the strict heterotroph, *Colpidium* (a positive priority effect). This facilitative priority effect of *P. bursaria* on *Colpidium* is absent in direct competition (Hsu et al., unpublished) and is unlikely due to free-living endosymbiotic algae *Chlorella*. Although *Chlorella* can escape host cells and grow independent of *P. bursaria* (Lowe et al., 2016), there was no significant increase in chlorophyll-*a* from free-living *Chlorella* across light levels (**Supplementary Figure S2**) perhaps due to the presence of *Chlorella* viruses in our cultures or lack of regulatory factors to survive on its own (Kato and Imamura, 2009; Johnson, 2011). Facilitative priority effects are often explained by niche modification, in which the early arriving species alters the type of niches available in the local environment, consequently shaping the species that are capable of colonization (Fukami, 2015). One mechanistic explanation could be that photosynthetic activity by early arriving *P. bursaria* increased available organic material in the system, increasing the bacterial resources and, ultimately, the maximum population size of *Colpidium*. Because *Chlorella* are endosymbiotic, such a release of organic matter would have to occur through either excretion of waste products by *P. bursaria*, or lysis of *P. bursaria* cells and subsequent remineralization of this organic matter by bacteria. Positive priority effects have also been observed when certain plant species increase nutrient availability and alter soil chemistry, facilitate the establishment of mutualistic mycorrhizal networks, or leave behind nutrient-rich litter (D'Antonio and Vitousek, 1992; van de Voorde et al., 2011). Species can also change the physical conditions of the environment to alter community succession. For example, grass invasions can promote seed germination of other alien grasses by changing soil temperature and moisture (D'Antonio and Vitousek, 1992).

In contrast, early arrival by *Colpidium* accelerated competitive exclusion of *P. bursaria* (a negative priority effect). In a previous study, Müller et al. (2012) cultured *P. bursaria* in *Colpidium* conditioned media, and found that allelochemicals produced by *Colpidium* did not inhibit *P. buraria* growth. Thus, the negative priority effects of *Colpidium* on *P. bursaria* may have been driven by competition for common resources,

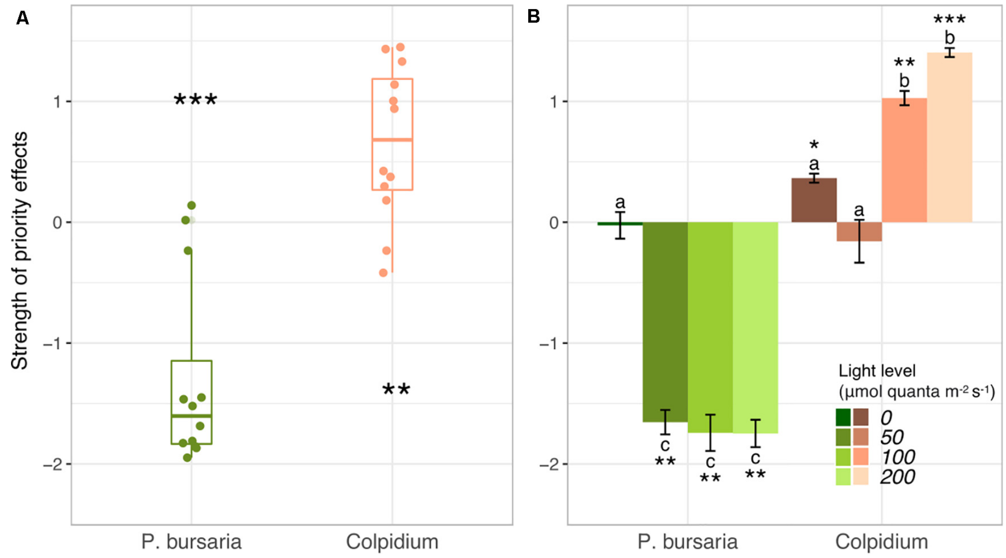

FIGURE 2 | Priority effects metric (Eq. 1) between *Colpidium* and *P. bursaria* (Vannette and Fukami, 2014) generated from population dynamics shown in **Figure 1**. **(A)** Boxplots of priority effects metric demonstrate that *Colpidium* was positively affected by early arriving *P. bursaria*, and *P. bursaria* was negatively affected by early arriving *Colpidium.* **(B)** The strength of priority effects by light level. Different letters represent statistically significant differences at the $p < 0.05$ level (Tukey's HSD, analysis of variance comparing all light × species interactions, see summary of initial ANOVA tests **Supplementary Table S1**). On all panels, error bars represent ± one standard error. Stars indicate significant differences from zero (*T*-test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

such as bacterial prey, though we did not collect data on bacterial abundance as part of our study. *Paramecium bursaria* needs to maintain bacterial ingestion to provide important nutrients, such as nitrogen, essential for both *P. bursaria* and its endosymbiont, *Chlorella* (Albers and Wiessner, 1985; Johnson, 2011). This competition may be enhanced when *Colpidium* populations are pre-established. Indeed, niche preemption, in which the early arriving species reduces the resources available (Fukami, 2015), is a widely known phenomenon observed in other microbial systems (Peay et al., 2012; Ng et al., 2013; Vannette and Fukami, 2014), as well as between competing plants (Grman and Suding, 2010; Kardol et al., 2013) and animals (Geange and Stier, 2009).

The availability of benefits from metabolic symbiosis shapes the strength and direction of priority effects. In our study, the photosynthetic contribution of *Chlorella* was limited by light availability: In light environments (>0 μmol quanta m^{-2} s^{-1}) when symbiotic benefits were greatest, *P. bursaria* persisted for longer than in dark environments (0 μmol quanta m^{-2} s^{-1}). In other systems, the presence or absence of symbiotic partners has a similar effect. For example, in grassland systems where pioneer species preempt light and space niches from subsequent community members, the inclusion of soil mutualists (such as mycorrhizal fungi) reduced the strength of priority effects and allowed for the later establishment of native grass (Burkle and Belote, 2015).

In our study system, the metabolic value of symbiosis (acquired phototrophy) should scale with light availability. We found that the strength of positive priority effects (facilitation of *Colpidium*) increased with increasing light, which supports our proposed mechanism: that *Colpidium* is facilitated by increased bacterial growth supported by *P. bursaria* symbiont photosynthetic activity. Because photosynthetic rates (and population sizes of *P. bursaria* in control environments, **Figure 3A**) increase with increasing light levels, the supply of organic matter should also increase. However, the negative effect of *Colpidium* on *P. bursaria* did not scale with light. We found that increasing light (and, presumably, increasing photosynthetic benefits from acquired metabolism) did not alleviate competitive effects of *Colpdium* on *P. bursaria*, perhaps because *P. bursaria* initial growth (**Figure 3C**) and establishment were inhibited by the presence of established *Colpidium* populations, which are not light-dependent. This phenomenon may be explained by niche preemption, a mechanism of priority effects in which the early arriving species limits the resources available for successive species (Fukami, 2015). Pre-established *Colpidium* could have reduced bacterial densities, which limited *P. bursaria* population sizes across all light levels due to limited access to essential nitrogen. In this situation, additional photosynthate benefits of *Chlorella* were apparently insufficient to compensate for limited bacterial supply, especially since bacterial ingestion by *P. bursaria* is also a primary source of nitrogen for *Chlorella* (Lowe et al., 2016).

Although symbioses, such as metabolic symbiosis of *P. bursaria,* provide access to new resources and increased competitive ability, there are tradeoffs that constrain the survival in certain environments. *Paramecium bursaria* does not dominate *Colpidium* in low light environments, perhaps because it experiences tradeoffs between heterotrophy and photosynthesis (e.g., spatial constraints within its cytoplasm, where space is shared between digestive vacuoles and vacuoles containing *Chlorella* photosymbionts) that prevent *P. bursaria* from

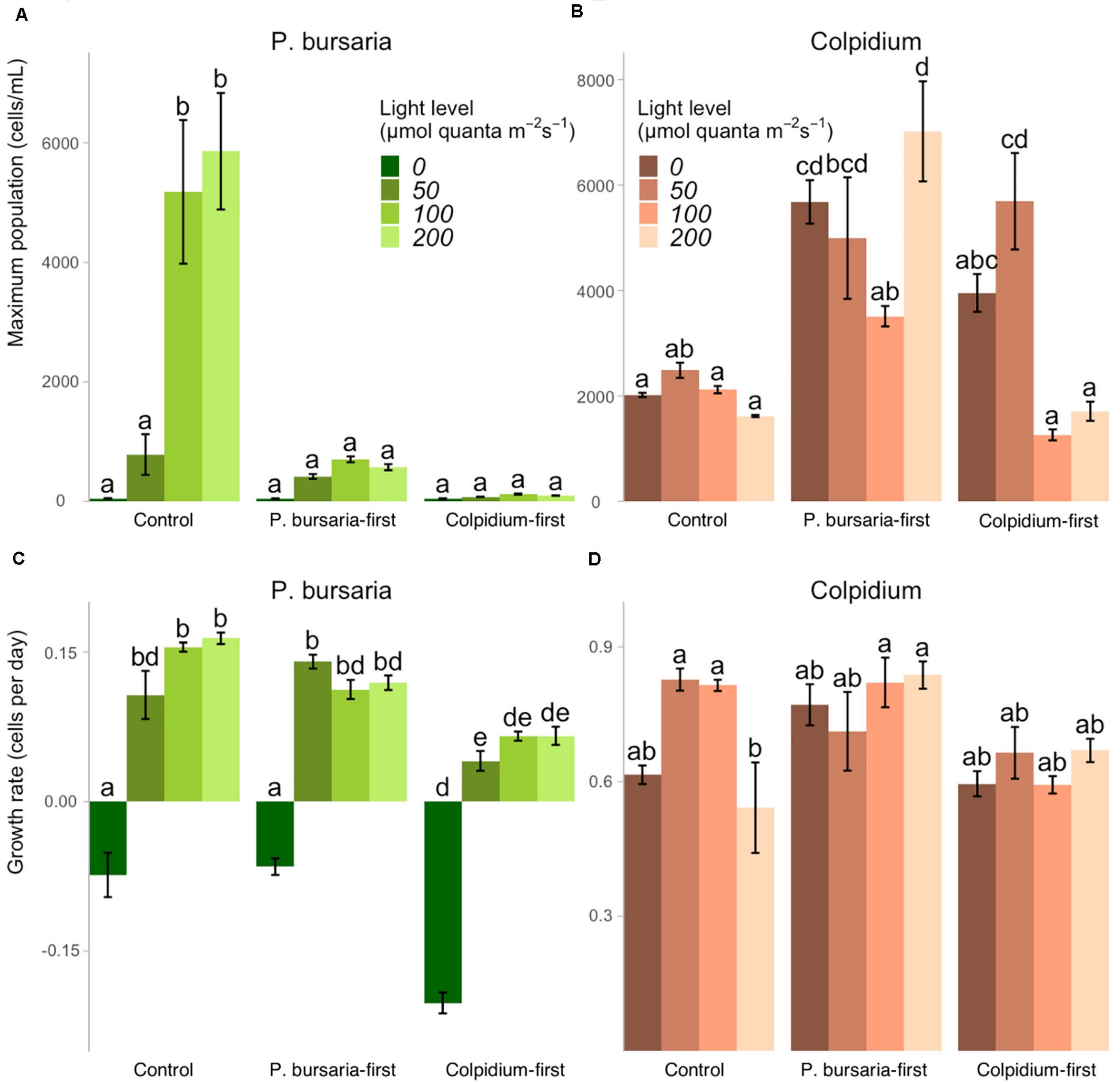

FIGURE 3 | The effect of light levels on maximum population size and growth rates for single-celled controls, *P. bursaria*-first and *Colpidium-first* treatments, using the species' observed light level response curves shown in **Figure 1**. Maximum population sizes for **(A)** *P. bursaria* demonstrates strong inhibition by early and late-arriving *Colpidium,* while maximum population sizes of **(B)** *Colpidium* generally benefited from presence of *P. bursaria.* The growth rates of **(C)** *P. bursaria* are significantly lower when *Colpidium* arrives first, while growth rates of **(D)** *Colpidium* are generally unaffected across treatments. Different letters represent statistically significant differences at the $p < 0.05$ level (Tukey's HSD within each panel, analysis of variance comparing all treatments × light levels, see summary of initial ANOVA tests **Supplementary Tables S2, S3**), and error bars represent ± one standard error.

persisting on heterotrophy alone (Salsbery and DeLong, 2018; Flynn et al., 2019). A previous model found that mixotrophs are most successful by being mostly photosynthetic and supplementing nutritional needs with heterotrophy (Crane and Grover, 2010), which could explain why the *P. bursaria* in our experiments were light-dependent. In darkness, endosymbiotic *Chlorella* cells may also become parasitic because they no longer provide photosynthates, and these costs may contribute to the accelerated declines in *P. bursaria* populations (Lowe et al., 2016). In other systems in which hosts rely on acquired photosynthesis, hosts have become obligately reliant on photosynthesis. For example, the acquired phototroph *Mesodinium rubrum* requires light for growth (Moeller et al., 2011), and zooxanthellate corals (which depend on symbiotic dinoflagellates for photosynthates) have decreased growth and survival without the symbiont (Brown, 1997; Douglas, 2003). Similarly, insects can have thermally sensitive bacterial partners that constrain the insects adaptation to elevated temperatures (Wernegreen, 2012).

Metabolic symbiosis allows species to access new resources that were previously unavailable. However, these benefits require energetic trade-offs that would become disadvantageous when environmental conditions change.

Because metabolic symbiosis alters competitive abilities and priority effects, it has cascading effects on the maintenance of diversity in ecosystems. In our system, acquired photosynthesis can facilitate the subsequent invasion by a second, heterotrophic species, and facilitate long-term coexistence of *P. bursaria* and *Colpidium* (Hsu et al., unpublished). By using a stylized model system—protist microcosms whose light levels mimic a range of natural freshwater pond settings yet which contain a constrained subset of species—we are able to more clearly identify the direction and strength of species interactions, including the facilitative effects of symbiosis. Previous work in grassland systems has shown that microbial symbionts (e.g., mycorrhizae) can increase grassland biodiversity and ecosystem functioning via facilitative priority effects (Burkle and Belote, 2015). Long term studies show that wood-decaying fungi, metabolic symbionts of some insects, can mediate positive priority effects of rare, wood-living beetles, therefore maintaining biodiversity of beetle species in boreal forests in Sweden (Weslien et al., 2011). Thus, accounting for metabolic symbiosis allows us to better predict the outcomes of competition, strength of priority effects, and success of restoration projects. The results of our study demonstrate the importance of metabolic symbiosis on competition outcomes under different community assemblage scenarios, and should encourage future work on priority effects and symbiosis.

DATA AVAILABILITY STATEMENT

All datasets and code used in this study can be accessed at https://doi.org/10.5281/zenodo.4342275. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

VH and HM conceived the ideas, designed methodology, analyzed the data, drafted the manuscript, contributed to subsequent revisions, and gave final approval for publication. VH collected the experimental data. Both authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by a UC Santa Barbara Undergraduate Research and Creative Activities grant to VH. Research was also sponsored by the U.S. Army Research Office and was accomplished under Cooperative Agreement No. W911NF-19-2-0026 for the Institute for Collaborative Biotechnologies. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the official policies, either expressed or implied, of the U.S. Government. The U.S. Government is authorized to reproduce and distribute reprints for Government purposes notwithstanding any copyright notation herein.

ACKNOWLEDGMENTS

We are grateful for laboratory assistance provided by M. Lepori-Bui. T. Fukami and C. Rodgers provided helpful discussion of experimental design and tools to quantify priority effects.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.614367/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.