ECOLOGICAL DEVELOPMENT AND FUNCTIONING OF BIOLOGICAL SOIL CRUSTS AFTER NATURAL AND HUMAN DISTURBANCES

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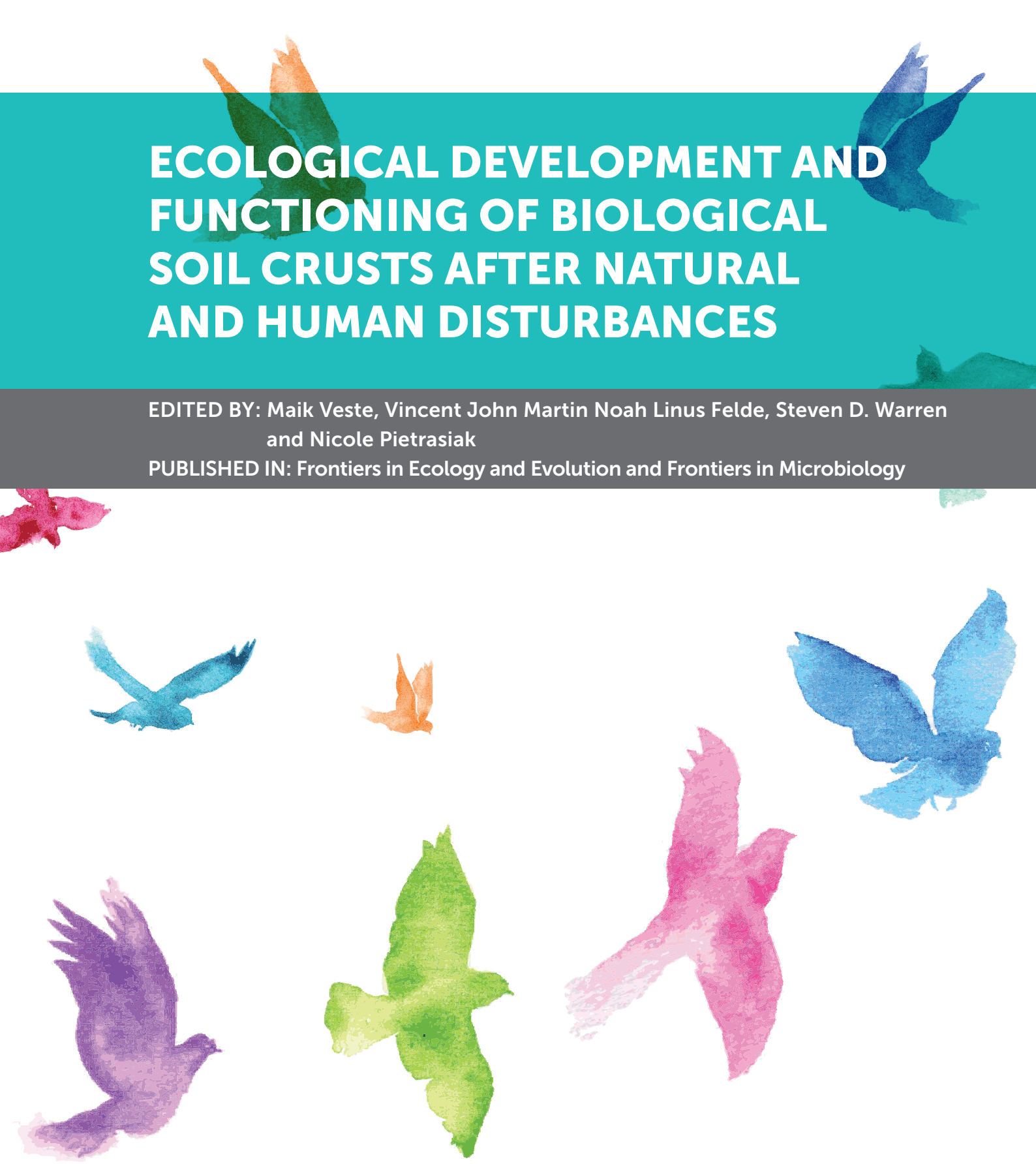

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ECOLOGICAL DEVELOPMENT AND FUNCTIONING OF BIOLOGICAL SOIL CRUSTS AFTER NATURAL AND HUMAN DISTURBANCES

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Table of Contents

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EDITORIAL
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Editorial: Ecological Development and Functioning of Biological Soil Crusts After Natural and Human Disturbances

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Editorial on the Research Topic

Ecological Development and Functioning of Biological Soil Crusts After Natural and Human Disturbances

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In recent years, the importance of soil health for ecosystem functions has come further into the scientific focus (Lehmann et al., 2020). Especially after severe ecosystem disturbances, soil formation has to start anew. Such disturbances, which reset ecosystem development to the starting point, can be of natural (volcanoes, mobile sand dunes, floods, glaciers) (La Farge et al., 2013; Lan et al., 2014) or human origin (post-mining landscapes, military training areas, agricultural lands) (Belnap et al., 2007; Schaaf et al., 2011). In these young ecosystems, the interactions between the initial colonizers, the inorganic matter, and the subsequent biogeochemical processes are an important prerequisite for the development of elemental fluxes, soil genesis, and thus for further ecosystem development. In addition to pioneer plants, photoautotrophic and heterotrophic microorganisms play a major role in the colonization of the freshly deposited sediment, regolith, or disturbed soil material. Biological soil crusts (BSCs) develop when various combinations of diminutive bacteria, terrestrial algae, fungi, lichens, and/or bryophytes occupy the upper few millimeters of the soil or regolith. They can be present in a wide range of ecological, including successional, and climatic conditions (**Figure 1**, Colesie et al., 2016) when and where disturbance and/or aridity have resulted in bare soil available for colonization. However, they are most prevalent in arid, semi-arid, and polar ecosystems and in temperate dry grasslands where vascular plant cover and diversity are characteristically low, leaving large areas available for colonization by some combination of the organismal groups mentioned above (Weber et al., 2016). The ecological roles of BSCs are numerous and diverse including the collection, accumulation, cycling of essential airborne and soil nutrients (Beraldi-Campesi et al., 2009), redistribution of precipitated water (Chamizo et al., 2016), soil formation and stabilization (Lan et al., 2014). Various ecological studies on the development and ecosystem functioning of BSCs have been conducted during the last three decades (Belnap and Lange, 2003; Breckle et al., 2008; Weber et al., 2016). The nexus of science related to BSCs and aerobiology strongly suggests that BSCs can become established or re-established *via* naturally occurring processes (Condon et al., 2020), as BSC propagules are found naturally in the atmosphere (Elliott et al., 2019), and can be transported very long distances between hemispheres,

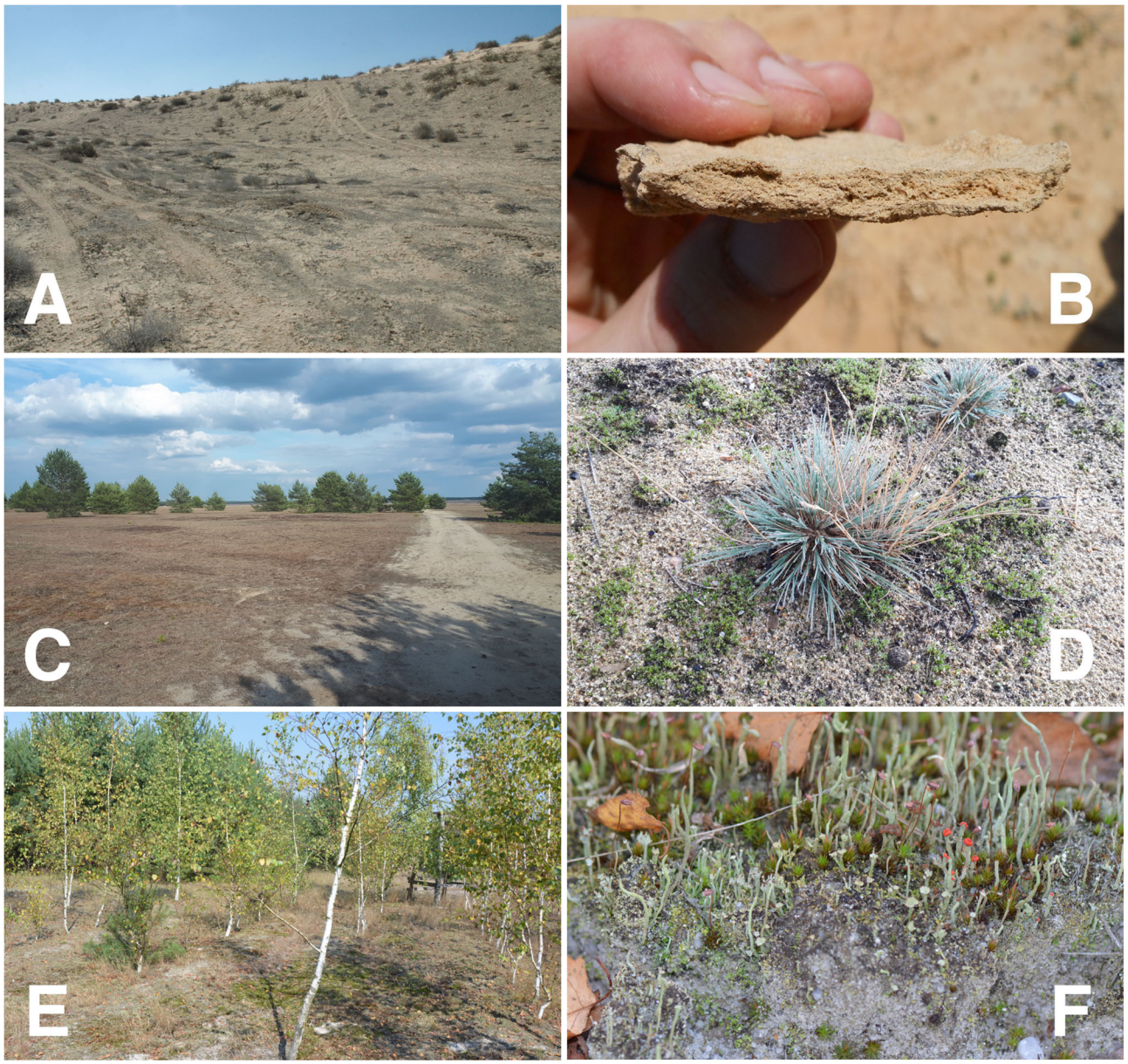

FIGURE 1 | Examples of different BSC in disturbed ecosystems **(A)** Biological soil crusts re-covered after disturbance by military vehicles in the Nizzana arid dunes of the north-western Negev, Israel, **(B)** Multi-layered cyanobacterial BSC in Nizzana, **(C)** Soil lichens- and moss-dominated BSC covering large areas of the sandy soils in a temperate dry grassland in a formerly disturbed military training area (Lieberoser Heide, Brandenburg, NE Germany), **(D)** Early successional stages of green algae and moss BSC established around gray hair-grass (*Corynephorus canescens*) in a temperate dry grassland, **(E)** Reclaimed post-mining site in Schlabendorf, Brandenburg, Germany is covered by soil lichen-moss communities mainly *Cladonia* spec. **(F)** (Gypser et al., 2015).

continents, and climates (Warren et al., 2019). Even after drastic disturbances, like fires, the relatively fast recovery of microorganisms can be observed (Dümig et al., 2014; Aanderud et al.; Chamizo et al., 2020). The ability of selected BSC organisms to recover quickly after disturbance can be exploited for various applications in ecosystem restoration (Gypser et al., 2015; Antoninka et al., 2020; Román et al., 2020). The speed and efficiency of inducing land surface stability (which is a prerequisite for soil development) depends on various environmental conditions (like texture and climate) (Chamizo et al., 2018) and may hence be very site specific. However, fast recovery is not always happen for all BSC organisms in all ecosystems. Under very harsh conditions such as the hyper-arid desert, this is not the case and recovery can be very slow. Here cyanobacteria and lichen biocrusts also need a longer time to establish after disturbance. All these studies in the different ecosystems underline the importance of the BSCs

for the biogeochemical and ecohydrological processes in the soil-plant system.

There remains a gap in understanding what shapes the globally heterogeneous biogeographical patterns of the different BSC organisms and how they relate to ecological processes surrounding the establishment, re-establishment, and development of BSCs. A more complete understanding of similarities and dissimilarities in the establishment, growth, and regeneration of BSCs from different geographical regions is essential to fully comprehend their contribution to ecosystem functioning and exploit their potential to improve the health of diverse soils. This is especially true for (remote) geographical regions that have hitherto been understudied, such as the Brazilian Caatinga, for which Szyja et al. emphasized the ecological importance of BSCs for providing ecosystem services.

In the recent decade, new research aimed to link the structure of BSC communities with ecosystem processes. Warren et al. provide an overview of how each organismal group contributes to the formation and maintenance of the structural and functional attributes of BSCs, how they reproduce, and how they are dispersed. The introduction of molecular biological methods (Keepers et al.) provided new insights into the function and composition of BSC communities in different ecosystems and climatic regions. Molecular biological studies in drylands in the Mojave Desert (Fisher et al.), Central Mexico (Becerra-Absalón et al.), Brazilian savanna (Machado-de-Lima et al.), and the arid lands of Western Australia (Autumn et al.) shed light on the composition of BSC communities and discussed their possible roles within their respective ecosystems. Pushkareva et al. examined differences in community composition and biomass in Arctic soil systems. An unusual human-made ecosystem is described by Sommer et al. that demonstrated how green algae, cyanobacteria, and diatoms can establish on severely saline mine tailings in Germany.

Still, the complex interspecific interactions between the organisms in the BSC communities and how these are influenced by the environment are not fully understood. Especially, the implication of heterogeneity and microhabitats on biogeochemical processes and plant-microbes (BSCs)—soil interactions need to be explored furthermore. In this context, Albright et al. show that microhabitat (e.g., vascular plant rhizosphere, BSC, and below BSC) was the strongest driver of differences in bacterial and fungal community richness, diversity, and composition in a dryland grassland in North America. The study by Condon and Pyke shows the importance of BSC organisms in plant communities of the semi-arid western US and argues that they should be included in ecological site descriptions due to their important contributions to ecosystem functions. The dominant components of biological crusts are photoautotrophic organisms, which primarily provide carbon for heterotrophs. More complex is the importance of the biological crusts for the N-cycling. Their ability to biologically fix N makes them an important N input pathway into nutrient-poor soils, and large amounts of inorganic N become accessible to the soil biota through the initial activity of the N-fixers. The interactive effects of BSC successional state and exposure to warmer temperatures on the rate and temperature sensitivity of soil heterotrophic C and N cycling in laboratory incubations could be shown in a mesocosm experiment under greenhouse conditions by Tucker et al.. The ecophysiological adaptation strategy and desiccation tolerance (Greenwood et al.) of BSC mosses are most crucial for their development in these extreme habitats, and furthermore during global warming. The study by Borchhardt and Gründling-Pfaff shows the ecophysiological plasticity of *Klebsormidium* to temperature in biological soil crusts from the Arctic and Antarctica.

Open topics for BSC research are still the interactions and feedbacks of biogeochemical processes between microorganisms and the soil-plant system. This applies particularly to the impact of climate change on the development of biological soil crusts.

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MV organized the Research Topic together with VF, SW, and NP. All authors contributed to the article and approved the submitted version.

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Differences in the Cyanobacterial Community Composition of Biocrusts From the Drylands of Central Mexico. Are There Endemic Species?

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In drylands worldwide, biocrusts, topsoil microbial communities, are prevalent, contributing to the biostabilization of soils and allowing the subsequent establishment and growth of vascular plants. In early successional biocrusts, cyanobacteria are the first dominant colonizers of bare ground, largely determining their functioning. However, there are large gaps in our knowledge of the cyanobacterial diversity in biocrusts, particularly in understudied geographic regions, such as the tropical latitudes. We analyzed the diversity of the cyanobacteria inhabiting the biocrusts of semideserts from Central Mexico in two localities belonging to the same desert system (Chihuahuan Desert) that are separated by a cordillera that crosses the center of Mexico. Morphological identification of the cyanobacteria was carried out after cultivation in parallel with the direct observation of the environmental samples and was supported by genetic characterization through analysis of the 16S rRNA gene of the isolated strains and by next-generation sequencing of the soil samples. Taxonomic assignment revealed a clear dominance of heterocystous cyanobacteria at one of the studied locations (Actopan, Hidalgo state). Although heterocystous forms were abundant at the other location (Atexcac, Puebla state), almost a third of the cyanobacterial phylotypes were represented by unicellular/colonial cyanobacteria, mostly *Chroococcidiopsis* spp. Only 28.4% of the phylotypes were found to be common to both soils. Most of the other taxa, however, were biocrust-type specific, and approximately 35% of the phylotypes were found to be unique to the soil they were collected in. In addition, differences in the abundances of the shared cyanobacteria between the locations were also found. These differences in the cyanobacterial distribution were supported by the distinct responses of the isolated strains representative of the sites to extreme heat and desiccation in bioassays. Some cyanobacteria with high abundance or only present at the hottest Actopan site, such as *Scytonema hyalinum*, *Scytonema crispum, Nostoc commune*, *Nostoc* sp., and *Calothrix parietina*, survived extreme heat and desiccation.

However, *Tolypothrix distorta* and *Chroococcidiopsis* spp. were clearly sensitive to these extreme conditions in relation to their lower abundances at Actopan as opposed to Atexcac. Since novel biocrust-associated phylotypes were also found, the emergence of endemic cyanobacterial taxa is discussed.

Keywords: biological soil crusts, cyanobacteria, Illumina sequencing, drylands, endemic species

INTRODUCTION

Biological soil crusts (biocrusts) are assemblages of different organisms (principally cyanobacteria, heterotrophic bacteria, algae, fungi, lichens, and bryophytes) that develop in dryland ecosystems compromising the topsoil layer, in which soil particles are aggregated through the presence and activity of these biota, and the resulting living crust covers the surface of the ground in the interspaces between plants (Garcia-Pichel, 2002; Belnap and Lange, 2003). Cyanobacteria have been proposed to act as pioneers in the stabilization process of these soils (Garcia-Pichel and Wojciechowski, 2009), with the production of polysaccharide sheaths by some of them, that aid in the formation of often centimeter-long filament bundles, contributing to the creation of a layer of fines and further enabling secondary colonization by heterocystous cyanobacteria, with eventual colonization by lichens or mosses (Garcia-Pichel et al., 2016). Soil characteristics, mainly texture, greatly influence biocrust formation and structure since a fine texture allows motility of colonizing filaments, further increasing soil aggregation (Rozenstein et al., 2014; Chamizo et al., 2018). The physiological features of different cyanobacteria will determine the colonizing succession within a cyanobacterial biocrust community, since those populations able to cope to extreme conditions, such as desiccation, temperature extremes, and high radiation, will survive in these unfavorable environments, such as the drylands. Many of these traits are related to adaptive mechanisms, for instance, the synthesis of sunscreen pigments or dormant cells (akinetes) that allow to cope with high radiation and desiccation respectively (Garcia-Pichel and Castenholz, 1991; Hu et al., 2012). Evidence has shown that cyanobacteria from biocrusts play important roles in key ecosystem processes, such as their contributions to soil fertility through nitrogen fixation and carbon sequestration, their ability to stabilize soils, their response to and recovery from fire and surface disturbance, and their effects on vascular plant establishment and growth (Lange and Belnap, 2016). Nitrogen-fixing cyanobacteria, free or growing in symbiosis in lichens, are the dominant N fixers in biocrusts (Barger et al., 2016), which exert a strong influence on the enrichment of the N pool in soils from low-nutrient environments.

Biocrusts are found on all continents, and studies of their distribution have expanded in North America, Asia, Africa, the Middle East, and Europe (Bowker et al., 2016 and references therein). Knowledge about cyanobacteria is rapidly increasing with the use of culture-independent sequencing studies. For instance, a continental-scale compositional survey of the cyanobacterial diversity in biocrusts of geographically distinct areas in the arid western United States revealed patterns of its abundance and distribution (Garcia-Pichel et al., 2013). Other studies showed differences in the cyanobacterial populations depending on the geographical region. In different arid and semiarid regions of warm climates, the filamentous non-heterocystous genus *Microcoleus* is the major component of the biocrusts (Büdel et al., 2016), while in Western Europe and Artic biocrust other filamentous non-heterocystous cyanobacteria belonging to the family of Leptolyngbyaceae were found to be dominant (Pushkareva et al., 2015; Williams et al., 2016). Less variation has been found regarding heterocystous cyanobacteria, whereby the genera *Scytonema*, *Nostoc*, and *Tolypothrix* have been found as dominant worldwide (Büdel et al., 2016). However, few investigations have been undertaken in temperate regions, and very little information is available from tropical latitudes (Belnap and Lange, 2003; Rivera-Aguilar et al., 2006; Büdel et al., 2009; Castillo-Monroy et al., 2011). In the central region of Mexico, there are arid and semiarid zones comprising important ecosystems, covering approximately 60% of the territory, in which biocrusts have a wide distribution that can constitute up to 70% of the drylands (Montaño and Monroy, 2000; Rivera-Aguilar et al., 2004). Therefore, in this work, two semidesert localities from Central Mexico were selected, one in Hidalgo state and another in Puebla state. These localities constitute the southern end of the oldest and most stable desert in Mexico, aged at approximately 50 million years old, the Chihuahua Desert. We aimed to study the differences in cyanobacterial diversity in biocrusts from these two locations, with distinct microclimatic conditions, through a polyphasic strategy in which microscopic analysis and molecular sequencing were combined with bioassays in order to identify causal factors influencing cyanobacterial species composition. This combined approach allowed us to determine the cyanobacterial diversity of biocrusts from one of many countries that is known for having extensive areas where these types of ecosystems are dominant, contributing to our understanding of the identity and distribution of these keystone microorganisms.

MATERIALS AND METHODS

Sites for Collection of Biocrust Samples

Biocrusts were collected from two semidesert localities from Central Mexico belonging to the extreme south of the Chihuahuan Desert (Rzedowski, 2006) (**Figure 1**). The first site was near the Actopan, Hidalgo state (20°16′02.9″N; 98°54′57.5″W) within the Mezquital Valley, and the second location was around the crater lake of San Luis Atexcac, Puebla state (19°20′13″N; 97°21′19″W) (**Figure 2**). Basaltic volcanic flows and mountains (**Figure 2A**) separate the two sites which are located in tropical latitudes. However, due to the altitude

FIGURE 1 | (A) Location of sampling sites, **(B)** general view of Actopan locality in Hidalgo state, **(C,D)** biocrusts from Actopan, **(E)** general view of Atexcac locality in Guadalupe Victoria, Puebla state, **(F,G)** biocrusts from Atexcac.

at which they are located, more than 2,000 m.a.s.l., they are considered temperate zones. The rainfall regime is different from that of the tropical latitude due to the development of orographic rain shadows (Rzedowski, 1978) so the climate is semiarid (**Figures 2B,C**). There are two main seasons, a warmer one, from May to October and a cooler one, during the rest of the year. In the warmest month (May), the mean monthly temperature in Actopan was 19.1°C (average data from 1981 to 2010), and the minimum and maximum average temperatures ranged from 9.5 to 28.7°C; while in Atexcac, this was 17.0°C, and ranged from 7.7 to 26.3°C. In the coldest months (January and December) the mean monthly temperature in Actopan was 12.4°C and ranged from 2.5 to 22.2°C, while in Atexcac this was 11.2°C and ranged from 1.3 to 21.1°C. The rainfall mainly occurs in the warmer season, normally in June and September. The Actopan location presented a mean annual temperature (MAT) of 16.4°C, the minimum and maximum mean annual temperatures were 7.8°C and 25.1°C, respectively, and the minimum and maximum temperatures recorded on the coldest and warmest days were −7°C and 47°C, respectively. The mean annual precipitation (MAP) was 436 mm, and the precipitation ranged from 400 to 900 mm. The soil type was a Phaeozem (Mollisol), fine-textured, with both alluvial and andesita deposits, and the vegetation was xerophytic crassicaule shrubs (Mesquite shrubs and trees), dominated by *Prosopsis* spp. with crassicaule (fleshy stemmed) plants. The Atexcac location had an MAT of 13.9°C, the minimum and maximum annual temperatures were 5.5°C and 22.4°C, respectively, and the minimum and maximum temperatures recorded on the coldest and warmest days were −12°C and 36°C, respectively. The MAP was 372 mm, with a precipitation range of 400–900 mm. The soil type was Phaeozem calcareous, fine-textured, and the vegetation was xerophytic shrubs with Izotal, dominated by *Yucca* and other Agavoideae (Alcocer et al., 2004). Another important climatic difference between the sites was the insolation; in Actopan, the location receives greater insolation all year round than does Atexcac (**Figures 2G,H**). The environmental data were obtained from government databases at Servicio Metereológico Nacional (SMN[1], accessed March 10, 2017), Instituto Nacional de Estadistica y Geografía (INEGI[2], accessed March 10, 2017), and Geoportal de la Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (Conabio[3], accessed March 10, 2017) and had been recorded over many years of monitoring (1951–2019). Daily climatic data of the SMN were obtained through the CICESE web platform[4].

Biocrust Sampling

Biocrust samples were collected in October (Actopan), and December (Atexcac) 2014 following the procedure previously described (Garcia-Pichel et al., 2013; Muñoz-Martín et al., 2019): Nine biocrust samples were taken in each site, within an area of 25–50 m^2 that was inspected to choose places that had developed biocrusts. A preliminary determination of the major cyanobacterial morphotypes and their relative abundance was carried out by direct microscopy of wetted samples. Then, representative subsamples, according to microscopic inspection, and with equal size, were selected, mixed together and homogenized with a mortar and pestle, to form a composite sample. These multiple samples, integrated field patchiness of

[1]http://smn.cna.gob.mx/es/
[2]https://www.inegi.org.mx/
[3]http://www.conabio.gob.mx/informacion/gis/
[4]http://clicom-mex.cicese.mx

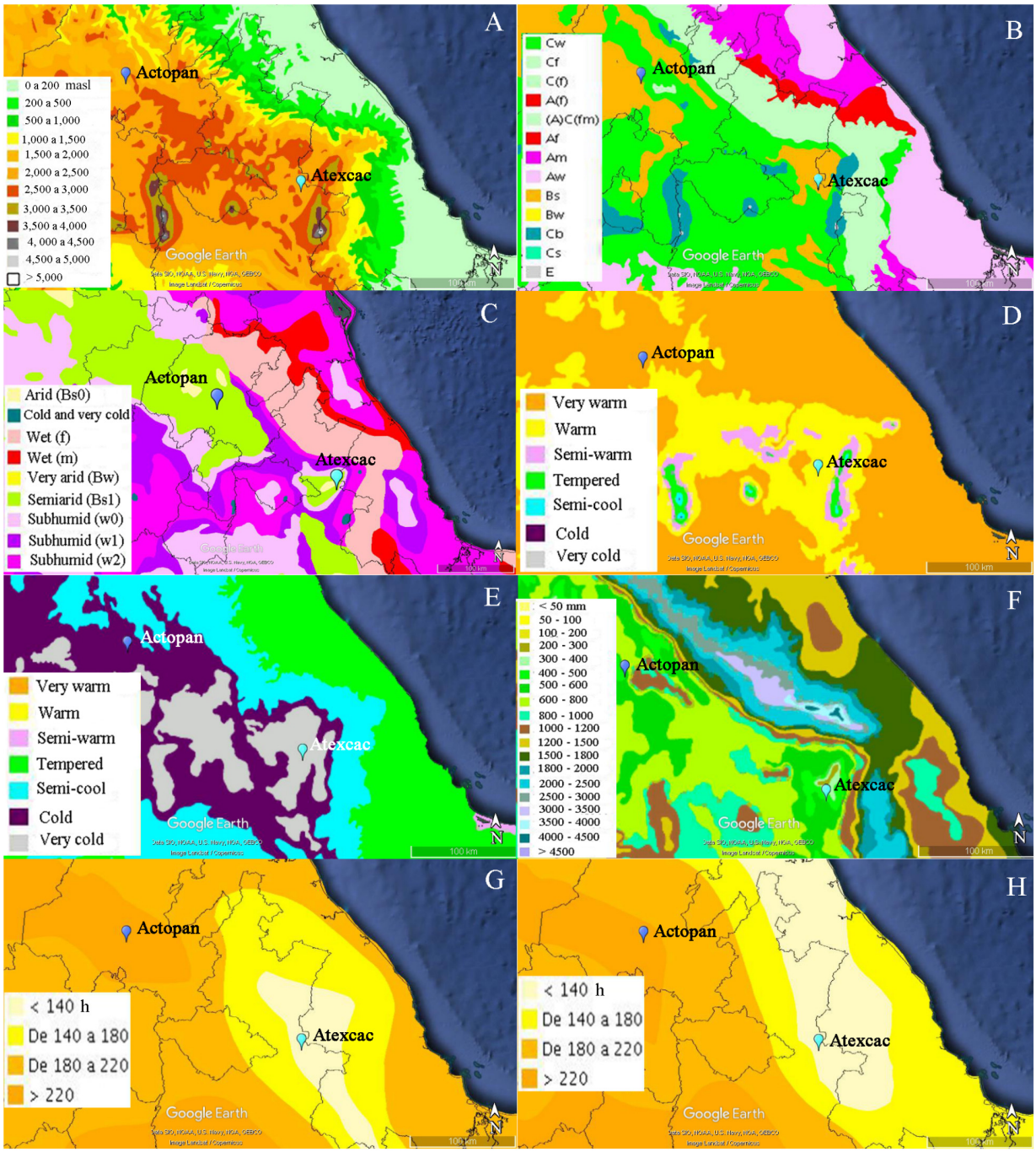

FIGURE 2 | Hypsometry and climatic characteristics of the two sampling sites: **(A)** hypsometry, **(B)** climate, **(C)** humidity, **(D)** maximum mean annual temperature, **(E)** minimum mean annual temperature, **(F)** mean annual precipitation, **(G)** hours of insolation during the month of maximum insolation (May), **(H)** hours of insolation during the month of minimum insolation (January). The climatic and geological data were obtained from the Geoportal of the National Commission for the Knowledge and Use of Biodiversity (CONABIO) in KML format and then imported into Google Earth (based on Image Landsat/Copernicus-Data SIO, NOAA, U.S. Navy, NGA, GEBCO) where the sites were located by means of the coordinates obtained with a GPS. masl, meters above sea level; Cw, temperate subhumid with dry winter; Cf, temperate humid; C(f), temperate without dry season; A(f), hot humid; (A)C(fm), semi-hot humid; Af, hot humid; Am, hot with summer precipitations; Aw, wet; Bs, semi-arid steppe; Bw, arid desert; Cb, temperate warm summer; Cs, temperate, dry summer; E, polar.

the communities (Pushkareva et al., 2015; Muñoz-Martín et al., 2019). The samples were collected using 60 mm Petri dishes, of which the plate bottom was inserted into the surface to excise a circular portion of the biocrust, collecting approximately 1 cm deep soil without disturbing the upper layer. After collection, the samples were stored in the dark at room temperature until use.

Strains Isolation and Culture Conditions

The dry biocrusts were reactivated by the addition of distilled water before their use for the isolation of strains. We isolated the strains by two methods: one by micromanipulation of the samples under the dissecting microscope with watchmaker's forceps, in which we manually isolated bundles of filaments as previously described (Garcia-Pichel et al., 2013) as well as filaments of heterocystous cyanobacteria. Seven strains were isolated by this procedure (see **Table 1**). The isolated bundles or filaments were then inoculated in multiwell plates with liquid BG11 (for non-heterocystous cyanobacteria) or $BG11_0$ medium (for heterocystous cyanobacteria) (Rippka et al., 1979).

TABLE 1 | Cyanobacterial strains isolated in this study.

Taxon	Strain number/culture collection no.	Sampling site	Figure
Nostoc commune	ACT709/UAM422	Actopan	**Figure 3A**
Nostoc sp.1	ACT703/UAM857	Actopan	**Figure 3B**
Nostoc sp.2	ACT732/UAM446	Actopan	**Figure 3C**
Tolypothrix distorta	ACT712/UAM443	Actopan	**Figure 3E**
	ATE705/UAM438	Atexcac	**Figure 3D**
	ATE717/UAM454	Atexcac	
Scytonema crispum	ACT685/UAM437*	Actopan	**Figure 3F**
Calothrix parietina	ACT696/UAM439	Actopan	**Figure 3G**
	ACT713/UAM858	Actopan	
Scytonema hyalinum	ACT694/UAM441	Actopan	
	ACT695/UAM447	Actopan	
	ATE698/UAM456	Atexcac	
	ACT699/UAM440*	Actopan	**Figure 3H**
	ACT700/UAM436	Actopan	
	ATE704/UAM455*	Atexcac	**Figure 3I**
	ACT706/UAM859*	Actopan	
	ACT711/UAM448*	Actopan	
	ATE714/UAM860	Atexcac	
Chroococcidiopsis sp.1	ATE715/UAM434	Atexcac	**Figure 3J**
Chroococcidiopsis sp.2	ATE707/UAM433	Atexcac	**Figure 3K**
Kamptonema sp.	ACT692/UAM457	Actopan	**Figure 3M**
Microcoleus vaginatus	ACT688/UAM445*	Actopan	**Figure 3N**
Porphyrosiphon notarisii	ACT693/UAM430*	Actopan	**Figure 3O**
Oculatella atacamensis	ATE710/UAM427	Atexcac	**Figure 3P**
Oculatella sp.	ACT687/UAM426	Actopan	**Figure 3V**
Leptolyngbya sp.	ACT691/UAM424	Actopan	**Figure 3T**
Leptolyngbya sp.	ACT689/UAM432	Actopan	**Figure 3U**
Leptolyngbya frigida	ACT684/UAM425	Actopan	**Figure 3Q**
Schizothrix sp.	ACT690/UAM431	Actopan	**Figures 3R,S**
Chroakolemma opaca	ACT686/UAM423	Actopan	
	ACT701/UAM861	Actopan	**Figure 3W**
	ACT702/UAM862	Actopan	
	ACT708/UAM863	Actopan	
Chroakolemma pellucida	ATE716/UAM429	Atexcac	
	ATE718/UAM428	Atexcac	
	ATE719/UAM864	Atexcac	**Figure 3X**
Synechococcus sp.	ATE729/UAM435	Atexcac	**Figure 3L**

**Strains isolated by micromanipulation (see "Materials and Methods" section).*

Cycloheximide (0.1 mg mL^{-1}) was also added to avoid fungal contamination. In a second, parallel method, the topsoil samples were ground with a mortar, and 0.1 g was mixed with 1.5 mL of cyanobacterial culture media and inoculated into Petri dishes (1.5% agar, with cycloheximide 0.1 mg mL^{-1}) with BG11 or $BG11_0$ media. The samples were incubated in a growth chamber at 28°C, 20–50 μmol photon m^{-2} s^{-1} and allowed to grow for approximately 4 weeks. Each strain was isolated from the colonies by selecting single trichomes or groups of cells for unicellular cyanobacteria using pulled capillary pipettes or forceps under a dissecting microscope (Leica, Leica Microsystems, Wetzler, Germany). The isolated strains were transferred to multiwell plates with liquid BG11 or $BG11_0$ and maintained under 28°C, 20–50 μmol photon m^{-2} s^{-1}.

Morphological Characterization

The isolated cultured strains and cyanobacterial populations in the environmental samples were analyzed for general morphological and morphometric characteristics as previously described (Becerra-Absalon et al., 2018). The characteristics of our cyanobacteria were compared with the information provided in the taxonomic keys of Komárek and Anagnostidis (1999, 2005) and Komárek (2013).

Isolation of Genomic DNA and Amplification of the 16S rRNA Gene of the Isolated Strains and an Environmental Sample

Total genomic DNA extraction of the isolated cultures and an environmental sample of a macroscopic colony of *Nostoc* was accomplished using the Ultraclean® Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, United States) with a modification previously described (Loza et al., 2013) to break the exopolysaccharides surrounding many of the cyanobacterial cells. This involved a three-cycle step that consisted of freezing 0.3 mL aliquots of cyanobacterial suspensions of each culture in liquid nitrogen, breaking them down with an adapted drill and melting them in a 60°C water bath.

The 16S rRNA gene was amplified by PCR using primer 27 as the forward primer (Wilmotte et al., 1993) and primer B23SR as the reverse (Lepère et al., 2000) under conditions previously described by Mateo et al. (2011) and following the PCR conditions of Gkelis et al. (2005). This reaction produced amplification fragments of approximately 2,000 bp that span the 16S rRNA gene and the intergenic region between the 16S and 23S rRNA genes. An agarose gel (1%) with a 1 kb gene ruler (MBL Biotools, Spain) and the fluorescent DNA stain Gel Red™ were employed to check if the amplification fragments had the correct size. The DNA was purified using Wizard SV Gel and PCR Clean-Up (Promega, Madison, WI, United States) and cloned into pGEM® -T Easy Vector Systems (Promega, Madison, WI, United States). The DNA from the colonies of recombinant clones carrying the correct-sized inserts was extracted using the Wizard Miniprep Kit (Promega). The sequencing of both strands was carried out at the Genomics Core Unit of the Spanish National Cancer Research Center, Spain. Several clones from

various strains were sequenced, obtaining a total of 46 nucleotide sequences which were deposited in the GenBank Database under the accession numbers: MK247967-MK248010 (isolated strains) and MK239479-MK239480 (environmental sample).

Phylogenetic Analyses of Sequence Data

The 16S rRNA gene sequences of approximately 1,500 bp were aligned and manually corrected using PhyDE-1 v0.9971 (Müller et al., 2010). A BLAST search (Altschul et al., 1990) was performed, and assignations with an identity value higher than 97.5% and other representative soil cyanobacteria sequences were downloaded from the NCBI database[5]. Multiple alignments of all these sequences, using *Gloeobacter violaceus* or *Chroococcidiopsis* spp. sequences as outgroups, were accomplished using the function ClustalW multiple alignment, and the alignment was later visually checked and corrected using PhyDE-1 v0.9971 (Müller et al., 2010). The phylogenetic trees were computed with MEGA version 7.0.21 (Kumar et al., 2016) using neighbor-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) algorithms. For NJ, the evolutionary distances were calculated by applying the Kimura 2-parameter, gamma distributed and invariant sites with a pairwise deletion of gaps and missing data. The distances for the ML tree were estimated by the Kimura 2-parameter, gamma distributed and invariant sites, assuming a gamma distribution with four categories with the nearest-neighbor-interchange. The MP tree was built with the subtree-pruning-regrafting search method with 10 initial trees and 3 search levels. The gaps and missing data were treated with the complete deletion option. The standard error in all analyses was estimated with the bootstrap phylogeny test (Felsenstein, 1985) using 1,000 replications. The percentage of similarity between sequences was determined as (1-p-distance)*100.

Analyses of Cyanobacterial Community Composition by Amplicon Metagenomics

Amplicon metagenomics targeted to the 16S rRNA gene and Illumina MiSeq sequencing data were used to assess the diversity and community composition of the bacteria. One gram from each composite soil sample was aliquoted into four parts (0.25 g) for DNA extraction using a PowerSoil DNA extraction Kit (Mo Bio, Carlsbad, CA, United States), according to the manufacturer's instructions. Because many cells were difficult to lyse, an additional step was added at the beginning of the protocol as described in Muñoz-Martín et al. (2019): the soil was incubated with the homogenization solution and exposed to three freeze-thaw cycles, alternating immersion in liquid nitrogen, heating to 60°C, and homogenizing with a pellet pestle in an Eppendorf tube using a hand-operated homogenizer (Bosch, CSB-850-2RET). The DNA was eluted in 100 μL of buffer, and the four independent DNA extractions per sample were mixed for PCR amplifications. The V4 variable region from the 16S rRNA gene was amplified by PCR using the universal bacterial primers F515 and R806 as previously described by Caporaso et al. (2011) at the Microbiome Analysis Laboratory, Swette Center for Environmental Biotechnology, Biodesign Institute of the Arizona State University (United States). The amplicons were processed using a MiSeq sequencer (Illumina) with a read length of 2 × 150 bp. At least 100,000 sequences were obtained for each amplicon. Quality control checks were performed on the raw sequence data using FastQC v 0.11.3. The sequence data were processed using QIIME v 1.9.0 (Caporaso et al., 2010) and the workflow described by Pylro et al. (2014), available on http://www.brmicrobiome.org/, based on UPARSE pipeline (Edgar, 2013) implemented by the software USEARCH v. 8.1, as described in detail by Muñoz-Martín et al. (2019). A similarity cut-off value of 97% was used to cluster the operational taxonomic units (OTUs). The OTU representative sequences were taxonomically assigned against the Greengenes database (13-08) (McDonald et al., 2011) first, using the RDP classifier method with a confidence value of 0.8 (Navas-Molina et al., 2013) to define the bacterial community composition at the phylum level. For more accurate taxonomic assignation of the cyanobacterial community, an approach similar to that described in Muñoz-Martín et al. (2019) was followed. First, the representative OTU sequences were matched against the 16S rDNA sequence database of the cultures obtained in this study, sequences from other cultures obtained by us from other biocrusts and other OTUs obtained in the abovementioned paper using the "uparse ref command" in USEARCH with the default parameters (Edgar, 2010). Then, all this information was compared with the taxonomic assignments made against the Greengenes database (McDonald et al., 2011) as mentioned above, and also against the SilvaMod database (Yilmaz et al., 2014) using the lowest common ancestor (LCA) algorithm implemented in CREST (Lanzen et al., 2012). In addition, the OTU representative sequences were Blasted against NCBI database. These steps allowed us to taxonomically assign almost all of the OTUs with a relative abundance of more than 0.1% in any of the locations. Alpha diversity indices (Chao1 estimator, Good's coverage and observed OTUs) were calculated using QIIME. Good's coverage estimates ranged from 99.73 to 99.80%, indicating that the large majority of the cyanobacterial diversity was captured. The OTU sequences have been deposited in the GenBank under accession numbers MK247058-MK247141. Raw sequencing data have been deposited in the NCBI Sequencing Read Archive under accession number PRJNA507723.

Cyanobacterial Survival Bioassays

To determine survival at high temperature and desiccation, a set of experiments was carried out with 23 of the isolated strains (see **Table 4** in the results). The survival was tested by two ways: the resistance (the ability of the cyanobacteria to withstand the extreme conditions), and the resilience (the ability of the cyanobacteria to recover following the extreme conditions). Therefore, first, bioassays were carried out at 40°C in liquid BG11 or $BG11_0$ (for non-heterocystous and heterocystous cyanobacteria, respectively) (Rippka et al., 1979) with equal amounts of inoculum from each of the strain, in duplicate; strains were distributed in wells of sterile polystyrene 6-well microtiter plates (IWAKI Microplate). They were incubated for 25 days in a 16:8 h light:dark period with an irradiance of 30 μmol photon $m^{-2}s^{-1}$. After this period of time, the cultures were left in these

[5]http://www.ncbi.nlm.nih.gov/blast

conditions until total desiccation and maintained thereafter for a year. To test the resilience following these conditions, culture medium was added to the desiccated strains and then the samples were maintained at room temperature (22–24°C). The retention or total loss of pigmentation was tested for these bioassays, using the retention of chlorophyll a as a proxy of the survival, as previously described (Garcia-Pichel et al., 2013; Zhou et al., 2016).

RESULTS

Morphological and Molecular (16S rRNA Gene) Analysis of Cultures: Polyphasic Identification of Isolated Strains

A total of 37 strains were isolated from the biocrust samples and cultured (**Table 1**); these strains were phenotypically and phylogenetically characterized (**Figures 3**, **4**). In addition, an environmental sample of a macroscopic colony of *Nostoc* found at the Actopan sampling site and was also characterized to compare with the *Nostoc* isolated strains (see below). The combined morphological and genetic evaluation allowed us taxonomic identification of 21 cyanobacterial species (**Table 1**), in which, strains corresponding to the same species showed similar morphological characteristics (data not shown). Eighteen of the strains belonged to the heterocystous cyanobacteria (**Figures 3A–I**, **4A**), and 19 were non-heterocystous, of which most were filamentous (16 strains), while only three strains belonged to unicellular/colonial cyanobacteria (**Figures 3J–X**, **4B**). Nine clusters were identified in the phylogenetic tree of the heterocystous cyanobacteria (**Figure 4A**) and corresponded to the morphotypes found (**Figures 3A–I**). The *Nostoc* isolates fell into three different clusters (cluster I, III, and VII) according to their distinct morphological characteristics

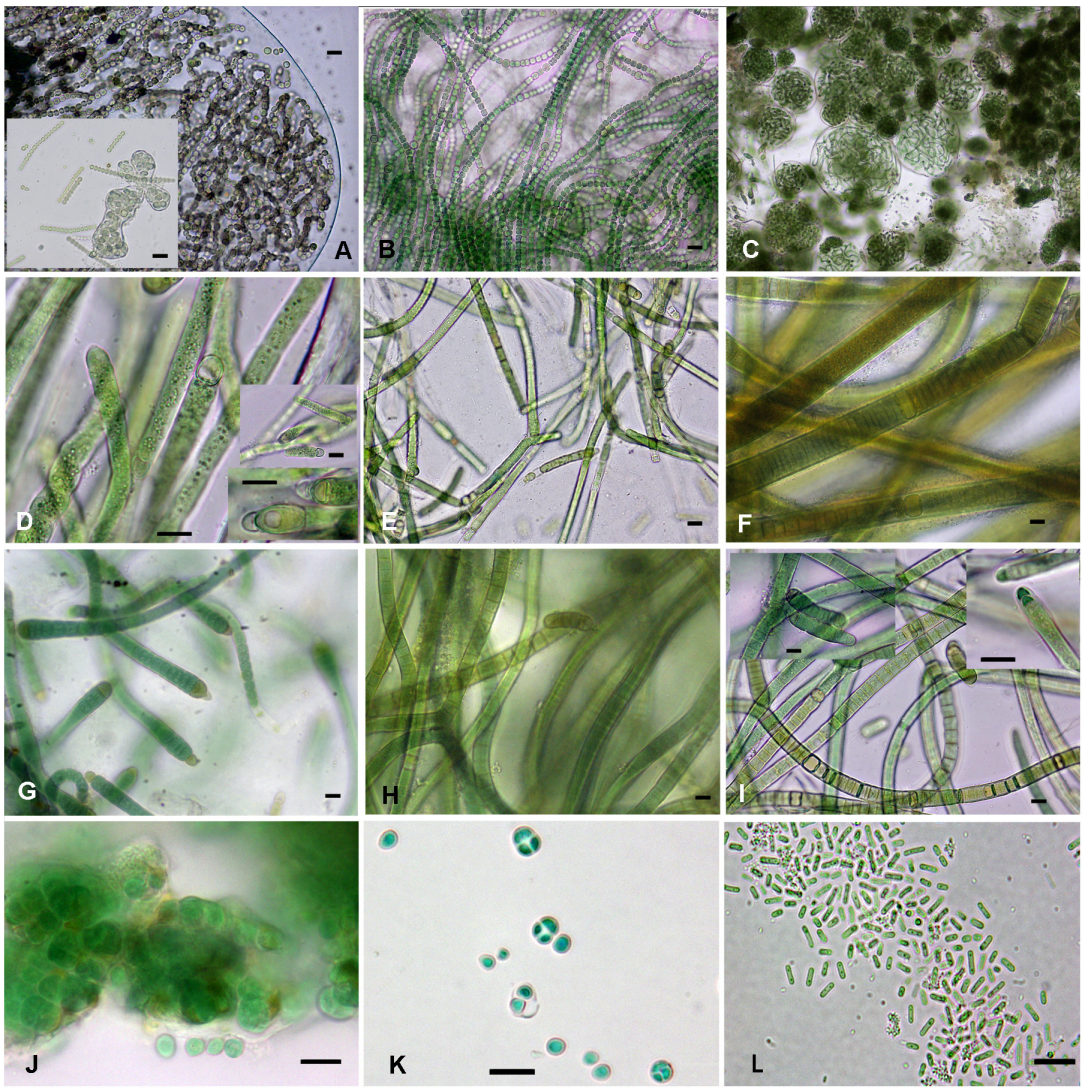

FIGURE 3 | Continued

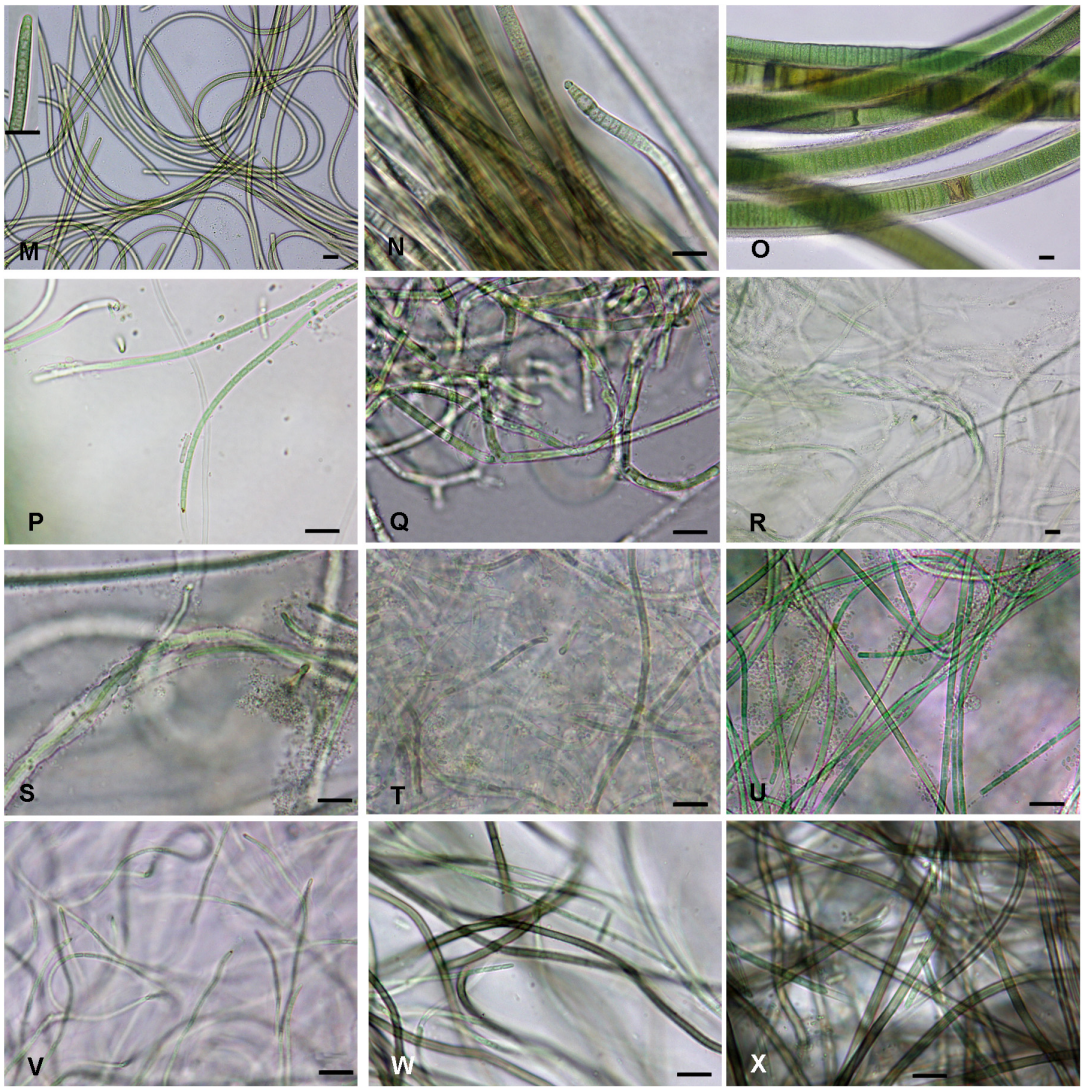

FIGURE 3 | Cyanobacterial isolated strains: **(A)** *Nostoc commune* ACT709, **(B)** *Nostoc* sp. 1 ACT703, **(C)** *Nostoc* sp. 2 ACT732, **(D)** *Tolypothrix distorta* ATE705, **(E)** *Tolypothrix distorta* ACT712, **(F)** *Scytonema crispum* ACT685, **(G)** *Calothrix parietina* ACT696, **(H)** *Scytonema hyalinum* ACT699, **(I)** *Scytonema hyalinum* ATE704, **(J)** *Chroococcidiopsis* sp.1 ATE715, **(K)** *Chroococcidiopsis* sp.2 ATE707, **(L)** *Synechococcus* sp. ATE729, **(M)** *Kamptonema* sp. ACT692, **(N)** *Microcoleus vaginatus* ACT688, **(O)** *Porphyrosiphon notarisii* ACT693, **(P)** *Oculatella atacamensis* ATE710, **(Q)** *Leptolyngbya frigida* ACT684, **(R,S)** *Schizothrix* sp. ACT690, **(T)** *Leptolyngbya* sp.1 ACT691, **(U)** *Leptolyngbya* sp.2 ACT689, **(V)** *Oculatella* sp. ACT687, **(W)** *Chroakolemma opaca* ACT701, **(X)** *Chroakolemma pellucida* ATE 719. Scale bar: 10 μm.

(**Figures 3A–C**). The 16S rRNA gene sequence from the *N. commune* isolate was placed in a cluster gathering of known representatives of *N. commune* from soils (cluster I). However, the sequences corresponding to the other isolated strains of the genus *Nostoc* (*Nostoc* sp. ACT732 and *Nostoc* sp. ACT703) were located in separate clusters, each of which included representatives of this genus, but with no clear specific identification (clusters III and VII, respectively). All the *Nostoc* phylotypes from the isolated strains were separated from those in cluster II, which included the sequence of the environmental sample fitting the morphological characteristics of *Nostoc indistinguendum* as well as other representatives of this taxon (cluster II). The well-supported group (cluster IV) corresponding to *Tolypothrix/Spirirestis* included our three isolates of *Tolypothrix distorta*, which showed typical characteristics of this genus, such as single falsely branched filaments originating below intercalary heterocysts (**Figures 3D,E**). Similarly, the morphological characteristics of the strain fitting the descriptions of *Scytonema crispum* (**Figure 3F**) were supported by its inclusion in a cluster together with other sequences of this taxon from the database (cluster V). Two isolates corresponding to *Calothrix* grouped with other *Calothrix* sequences from the databases corresponding to *C. parietina* (cluster VI); in addition, the morphological characteristics of these two isolates fit with

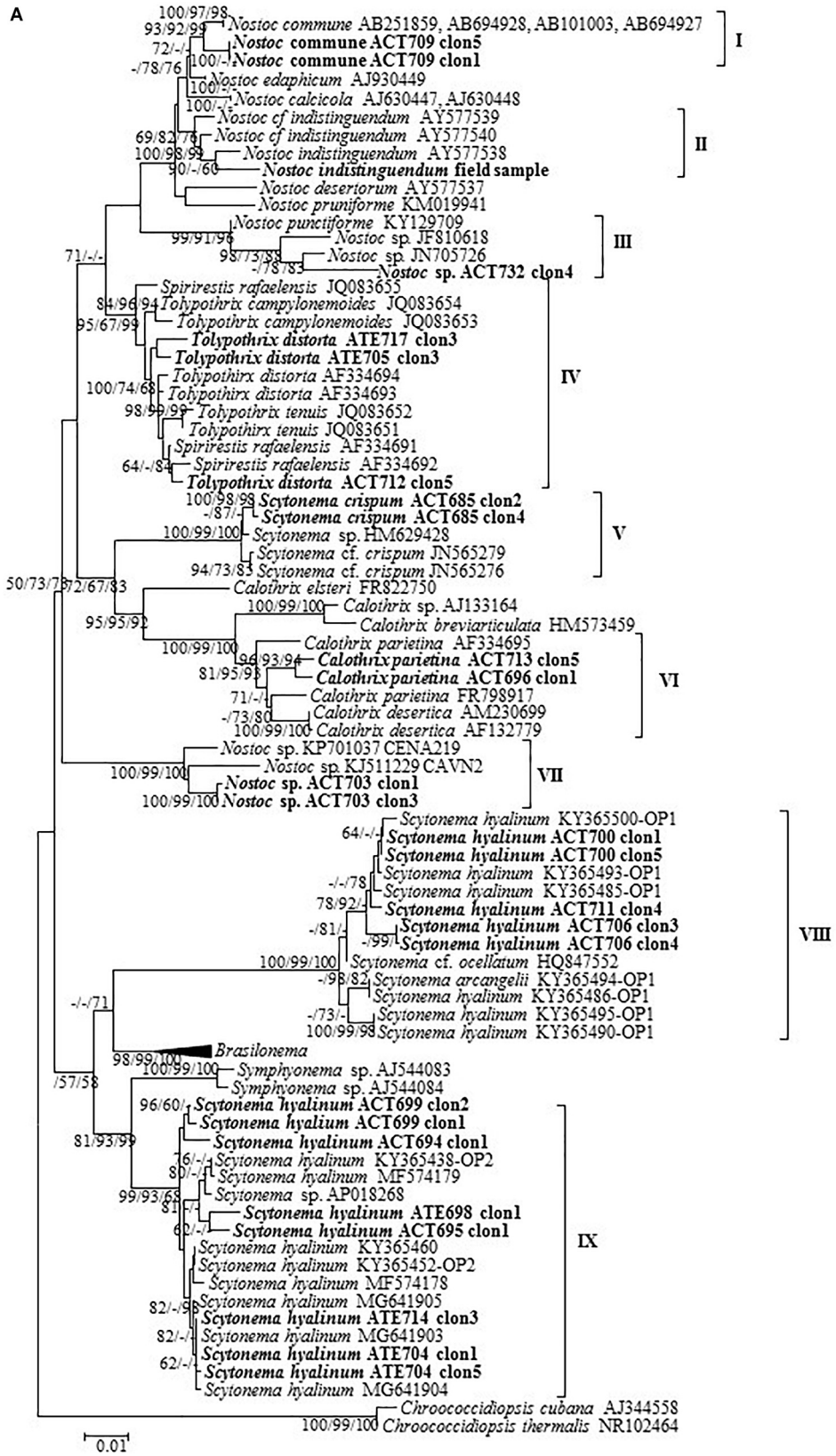

FIGURE 4 | Continued

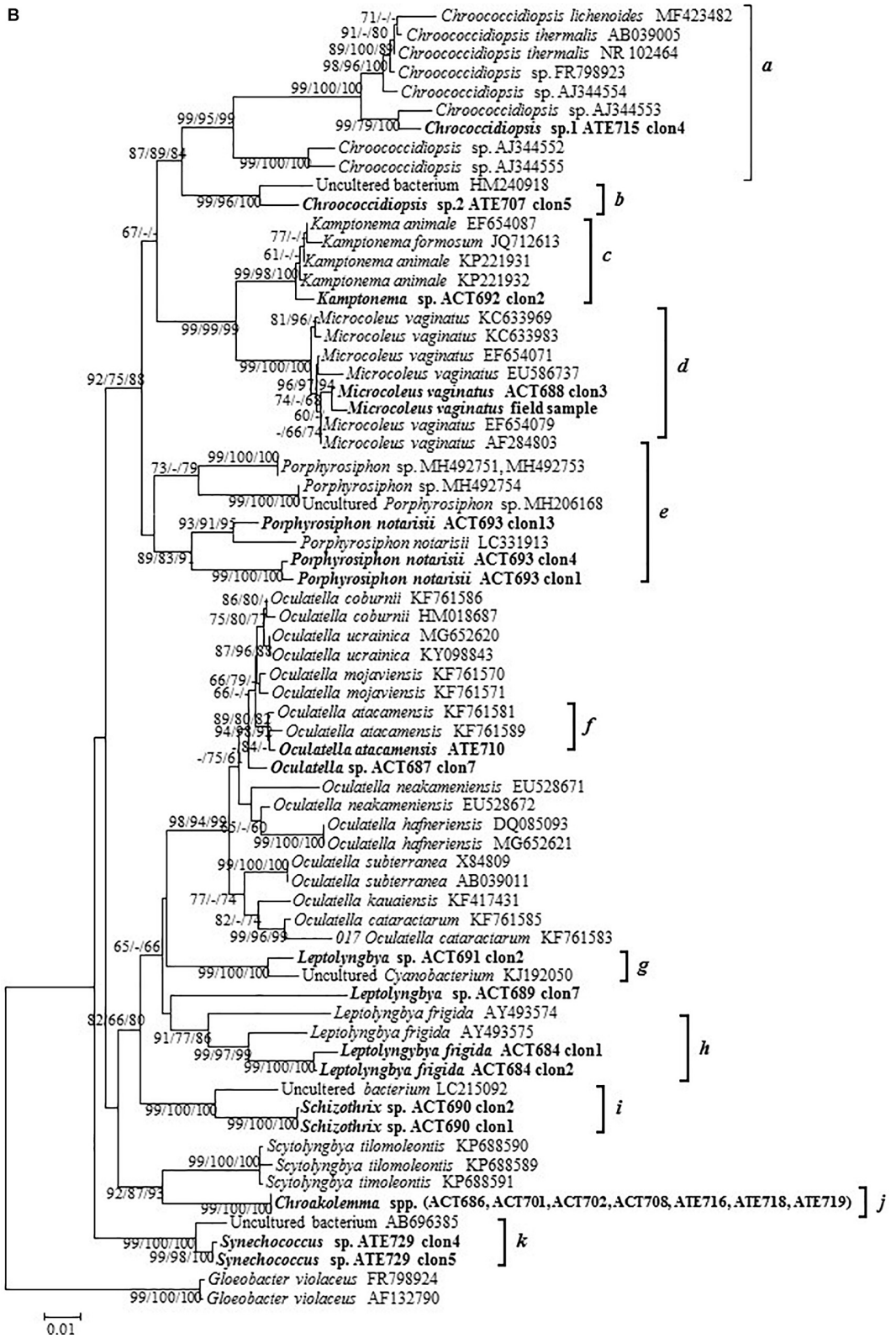

FIGURE 4 | Phylogenetic trees obtained by the neighbor-joining method representing **(A)** heterocystous cyanobacteria and **(B)** unicellular and filamentous non-heterocystous cyanobacteria, based on the analysis of the 16S rRNA gene, showing the position of the sequences obtained from the present study (in bold). Numbers near nodes indicate bootstrap values greater than or equal to 60% for NJ, ML, and MP.

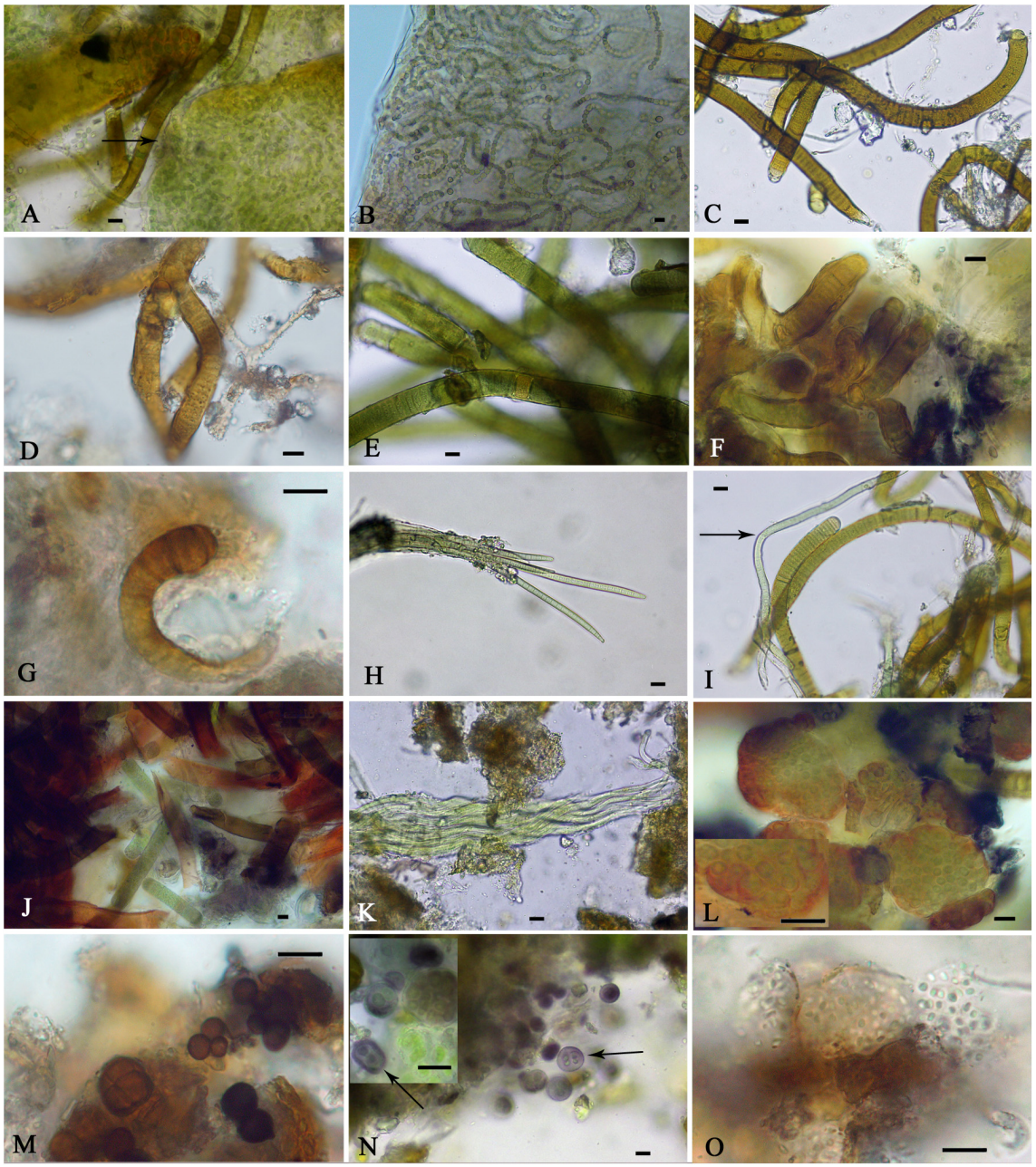

FIGURE 5 | Cyanobacteria observed in field samples: **(A)** *Nostoc commune* (black arrow), **(B)** *Nostoc indistinguendum*, **(C)** *Scytonema hyalinum*, **(D)** *Tolypothrix distorta*, **(E)** *Scytonema crispum*, **(F)** *Dapisostemon apicaliramis*, **(G)** *Calothrix parietina*, **(H)** *Microcoleus vaginatus*, **(I)** *Chroakolemma opaca* (black arrow), **(J)** *Porphyrosiphon notarisii*, **(K)** *Symplocastrum flechtnerae*, **(L)** *Aphanocapsa* sp., **(M)** *Chroococcidiopsis* sp.1, **(N)** *Chroococcidiopsis* sp.2 (black arrow), **(O)** *Chlorogloea* sp. Scale bar: 10 μm.

those of this taxon (**Figure 3G**). The *Scytonema hyalinum* isolates (**Figures 3H,I**) also showed typical morphology of the genus, grouping with other sequences of this species in different clusters (clusters VIII and IX) corresponding to two divergent 16S rRNA operons from single strains, as previously found (Yeager et al., 2007; Johansen et al., 2017; Muñoz-Martín et al., 2019).

Regarding non-heterocystous cyanobacteria, unicellular/colonial cyanobacteria were distributed in the phylogenetic tree among filamentous non-heterocystous cyanobacteria (**Figure 4B**) as previously found (Komarek et al., 2014). Clusters *a* and *b* included representatives of *Chroococcidiopsis* spp. (**Figures 3J,K**) together with sequences from this genus from databases (**Figure 4B**). The other coccoid cyanobacteria, which fitted into the genus *Synechococcus* (**Figure 3L**), were distantly placed from the *Chroococcidiopsis* spp. in the phylogenetic tree, with no matches found in the database (cluster *k*). Cluster *c*

grouped our isolate identified as *Kamptonema* sp. (**Figure 3M**), a new genus derived from the polyphyletic genus *Phormidium* (Strunecky et al., 2014), with other sequences of this genus (**Figure 4B**). Typical filamentous cyanobacteria, usually included in taxonomic descriptions from biocrusts, were *Microcoleus vaginatus* (**Figure 3N**, cluster *d*), *Porphyrosiphon notarisii* (**Figure 3O**, cluster *e*), *Oculatella atacamensis* (**Figure 3P**, cluster *f*), and *Leptolyngbya frigida* (**Figure 3Q**, cluster *h*). In addition, novel phylotypes and/or morphotypes were found in the studied biocrusts: the 16S rRNA gene sequence from a *Schizothrix* sp. isolate mapped alone in the tree (**Figures 3R,S**, cluster *i*) since no similar sequences were found in the database (the most similar sequence belonged to an uncultured bacterium with only 96% similarity). The genera *Leptolyngbya* and *Oculatella* were also represented by other sequences with no close relatives (**Figures 3T–V**, **4B**). Finally, seven of the isolated strains were recently characterized as belonging to a new genus: *Chroakolemma* (Becerra-Absalon et al., 2018) (**Figures 3W,X**, cluster *j*).

TABLE 2 | Cyanobacteria observed in field samples.

Taxon	Actopan	Atexcac	Figure
Nostoc commune	X	X	**Figure 5A**
Nostoc indistinguendum	X	X	**Figure 5B**
Scytonema hyalinum	X	X	**Figure 5C**
Tolypothrix distorta	X	X	**Figure 5D**
Scytonema crispum	X		**Figure 5E**
Dapisostemonum apicaliramis	X		**Figure 5F**
Calothrix parietina	X		**Figure 5G**
Microcoleus vaginatus	X	X	**Figure 5H**
Chroakolemma sp.	X	X	**Figure 5I**
Porphyrosiphon notarisii	X		**Figure 5J**
Symplocastrum flechtnerae	X		**Figure 5K**
Aphanocapsa sp.	X		**Figure 5L**
Chroococcidiopsis sp.		X	**Figure 5M**
Chroococcidiopsis sp.		X	**Figure 5N**
Chlorogloea sp.		X	**Figure 5O**

Microscopic Observation of Biocrust Field Samples

The identification of the isolated strains allowed us to better analyze the cyanobacteria observed in the soil samples; however, not all of the isolated strains were observed in these samples which may be due to their presence in relatively small amounts, and some of the cyanobacteria found in the samples could not be isolated. **Figure 5** shows representative micrographs of the cyanobacteria from the studied biocrusts, and **Table 2** shows the locations of the taxa in the samples. Most of the cyanobacteria found presented envelopes and pigmentation that were characteristic of cyanobacteria found in these extreme environments (**Figure 5**). Typical heterocystous cyanobacteria from biocrusts, such as *N. commune*, *N. indistinguendum*, *S. hyalinum*, and *T. distorta* (**Figures 5A–D**, respectively), were observed at both sampling sites (**Table 2**). However, other heterocystous forms, such as *S. crispum, Dapisostemon apicaliramis* and *Calothrix parietina* (**Figures 5E,F,G**, respectively), were observed only at the Actopan sampling site (**Table 2**). Regarding non-heterocystous cyanobacteria, only *M. vaginatus* (**Figure 5H**) and *Chroakolemma* sp. (**Figure 5I**) were observed at both sampling sites, while *Porphyrosiphon notarisii* (**Figure 5J**), *Symplocastrum flechtnerae* (**Figure 5K**) and *Aphanocapsa* sp. (**Figure 5L**) were observed in the Actopan samples. *Chroococcidiopsis* representatives (**Figures 5M,N**) and *Chlorogloea* sp. (**Figure 5O**) were only found at Atexcac (**Table 2**).

Molecular Analysis of Microbial Community Composition

The relative abundance of the dominant phyla of bacteria and plastids from the rRNA gene sequences of the studied biocrusts are displayed in **Figure 6**. The microbial phototrophs (cyanobacteria and algae) presented similar overall abundances in both samples (52%). However, this corresponded to a clear dominance of the cyanobacteria at Actopan (45.5%), while algae was the dominant phototroph community at Atexcac (38%). The rest of the biocrust bacterial rRNA gene sequences were also similar at both sampling sites; Proteobacteria and Bacteroidetes were the most abundant phyla (approximately 14% in both samples), followed by Actinobacteria (approximately 7%) and Acidobacteria (approximately 5–6%). Collectively, algae and all of these described taxa of bacteria accounted for >90% of the sequences recovered from these biocrusts.

Regarding cyanobacteria, clear differences were found in their taxonomic composition (**Figure 7**). The cumulative pie chart shows the distribution of the phylotype counts in which the heterocystous cyanobacteria are by far the most common members at Actopan (69.36%), while at Atexcac, to reach this percentage, it was necessary to combine the heterocystous cyanobacteria (41.03%) with the unicellular/colonial cyanobacteria (31.58%), mostly represented by phylotypes of *Chroococcidiopsis*. **Table 3** shows the taxonomic assignments of the OTUs based largely on comparisons with the sequences and the corresponding phenotypes of the isolated cultures in parallel with the microscopic analysis of the biocrust field samples. We could match sequences from the most abundant taxa (corresponding to OTUs 1–16) thanks to the collection of the isolated strains and/or after Blasting and comparing the results with the recorded morphotypes found at the sampling sites (**Table 2**). Clear differences in the observed taxa at each sampling site were found (**Figure 7**). **Figure 8** summarizes the data for all the OTUs together in a Venn diagram. Only 28.4% of the OTUs were found in both soils, while 33.9% were unique to the Actopan biocrusts and 37.6% were unique to the Atexcac biocrusts. In addition, differences in the relative abundance of the shared cyanobacteria in the samples were also found. *N. commune*, *S. hyalinum*, and *Microcoleus* sp. were more abundant at Actopan than at Atexcac, and some phylotypes, such as those corresponding to *S. crispum*, *N. indistinguendum*, *P. notarisii*, and other Oscillatorial, as well as two OTUs corresponding to *Nostoc* spp. were no present or almost undetectable (less than 1% of abundance) at Atexcac.

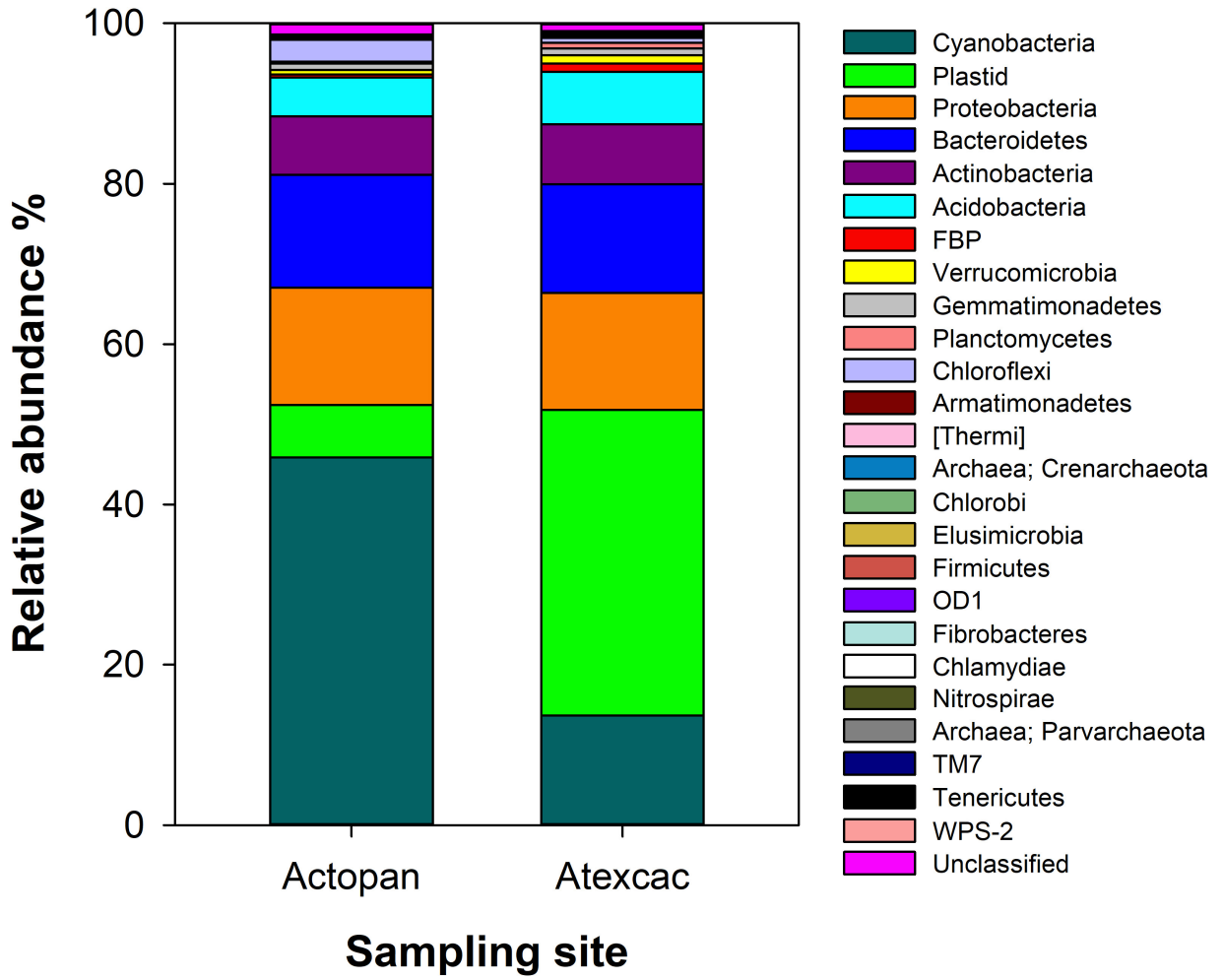

FIGURE 6 | Taxonomic microbial community composition from the studied biocrusts.

In contrast, *T. distorta*, *S. flechtnerae*, *M. vaginatus Nostoc* sp., *Oculatella* sp., and several OTUs corresponding to the *Chroococcidiopsis* presented higher abundances at Atexcac than at Actopan, with the percentages of the abundance in the second locality lower than 1%. Interestingly, some of the heterocystous cyanobacteria, although were present in low abundance, were present at only one of the sampling sites: *C. parietina*, *D. apicaliramis*, and other *Nostocal* were present only in Actopan, while *Mastigocladopsis* sp., *Stigonema* sp., *Macrochaete* sp., and *Desmonostoc* sp. were present only at Atexcac (**Figure 7**).

Sensitivity of Biocrust Isolated Cultures to Extreme Heat and Desiccation

Since climatic differences were found at the studied sampling sites, a series of bioassays were carried out to test the sensitivity of the cultured strains to extreme conditions. **Table 4** shows (i) the survival of 23 isolated cultures to extreme heat over 1 month, (ii) the subsequent response of the cultures to extreme heat and a year-long desiccation treatment, and (iii) the resilience of the cultures, following these extreme conditions, after the addition of culture medium to the desiccated cells. As expected, the majority of the cultures did not resist extreme heat, showing clear chlorosis within a range of 10–12 days for unicellular/colonial cyanobacteria and 10–20 days for filamentous non-heterocystous forms, except *Kamptonema* sp. which showed clear chlorosis starting on the fourth day of treatment. *Leptolyngbya* sp. survived after 25 days at 40°C but did not resist additional desiccation. Interestingly, the isolated strain belonging to the new genus *Chroakolemma* was the only non-heterocystous strain that survived the entire time tested. However, a variety of responses to extreme heat and desiccation were found for the heterocystous cyanobacteria. *S. crispum* and two strains of *S. hyalinum* and *C. parietina* survived the entire test period, but *T. distorta* did not survive after 19 days of culture at 40°C. Both *Nostoc* strains (*N. commune* and *Nostoc* sp.) survived 25 days of extreme heat but showed clear chlorosis after desiccation and extreme heat, and surprisingly recovered the pigmentation after addition of culture medium at room temperature showing resilience following heat and desiccation disturbance (**Table 4**).

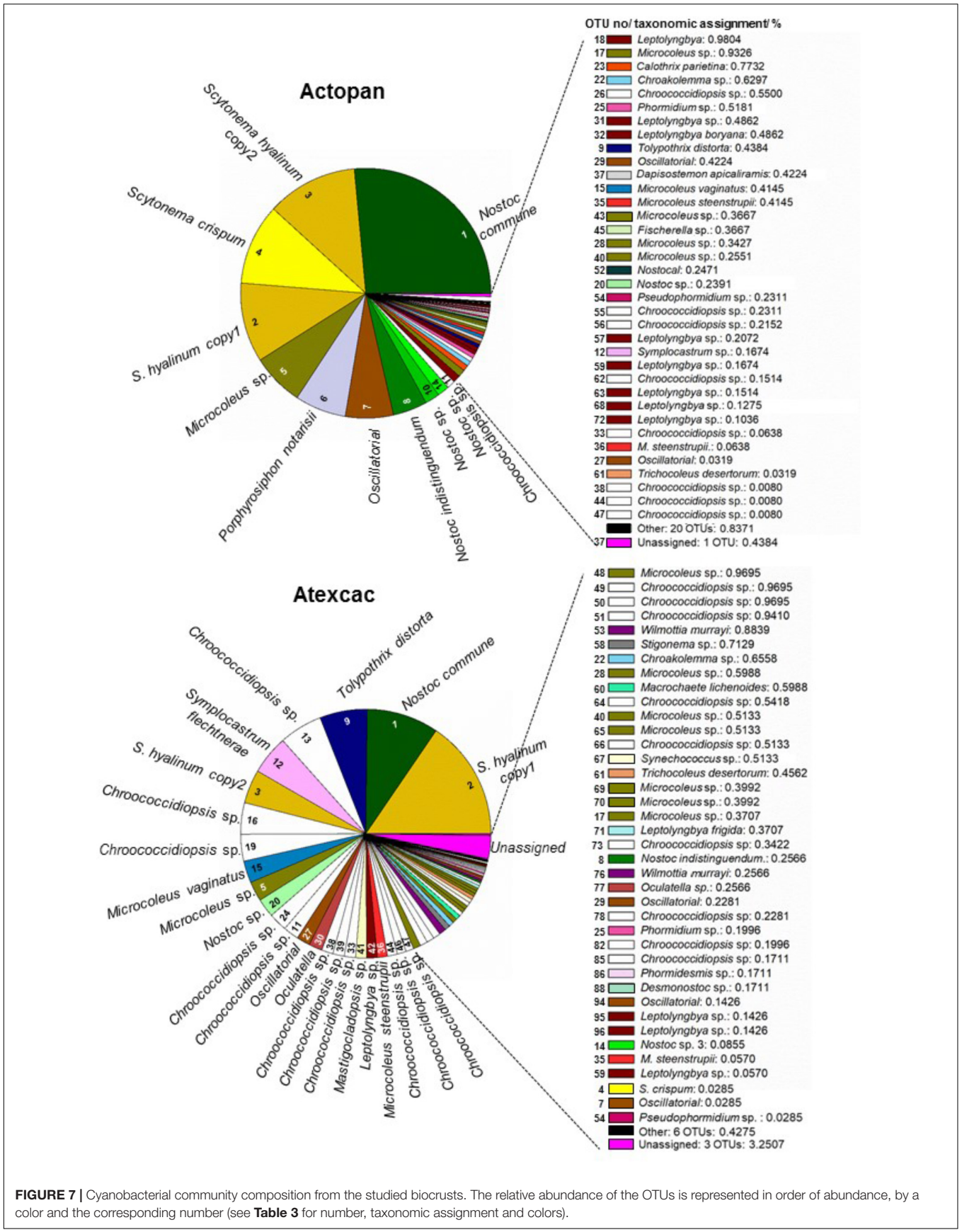

FIGURE 7 | Cyanobacterial community composition from the studied biocrusts. The relative abundance of the OTUs is represented in order of abundance, by a color and the corresponding number (see **Table 3** for number, taxonomic assignment and colors).

TABLE 3 | Taxonomic assignments of OTUs.

	OTU	Taxonomic assignment	Best match; accession no (%ID)/source	Observed in field sample	Isolated culture
	OTU1	*Nostoc commune*	***Nostoc commune* ACT709** (98)	X	X
	OTU8	*Nostoc indistinguendum*	**Unclustered *Nostoc indistinguendum*/field sample from Actopan** (100)	X	
	OTU10	*Nostoc* sp.	***Nostoc* sp. ACT703** (100)		X
	OTU14	*Nostoc* sp.	***Nostoc* sp. ACT732** (98)		
	OTU20	*Nostoc* sp.	*Nostoc* sp. LEGE 06158; HQ832947 (99)/Blast NCBI		
	OTU2	*Scytonema hyalinum*	***Scytonema hyalinum* ATE704 copy1** (100)	X	X
	OTU3		***Scytonema hyalinum* ACT700 copy2** (100)	X	X
	OTU4	*Scytonema crispum*	***Scytonema crispum* ACT685** (100)	X	X
	OTU9	*Tolypothrix distorta*	***Tolypothrix distorta* ATE705** (98)	X	X
	OTU23	*Calothrix parietina*	***Calothrix parietina* ACT696** (100)	X	X
	OTU37	*Dapisostemon apicaliramis*	*Dapisostemon apicaliramis*; KJ566945 (100)/Blast NCBI	X	
	OTU41	*Mastigocladopsis* sp.	*Mastigocladopsis* sp.; DQ235802 (99)/Blast NCBI		
	OTU45	*Fischerella* sp.	*Fischerella indica*; EU116033 (97)/Blast NCBI		
	OTU58	*Stigonema* sp.	*Stigonema spectabile*; KT867213 (98)/Blast NCBI		
	OTU60	*Macrochaete* sp.	*Macrochaete lichenoides*; KU559619 (99)/Blast NCBI		
	OTU88	*Desmonostoc* sp.	*Desmonostoc* sp.; MF770266 (99)/Blast NCBI		
	OTU52	Other Nostocales	Nostocales/Crest (Silva database) assignments		
	OTU11	*Chroococcidiopsis* sp.	*Chroococcidiopsis* sp.; AJ344555 (99)/Blast NCBI	X	
	OTU13		*Chroococcidiopsis* sp.; KU291459 (99)/Blast NCBI		
	OTU16		*Chroococcidiopsis* sp./Crest (Silva database) assignments		
	OTU19		*Chroococcidiopsis* sp./Crest (Silva database) assignments		
	OTU24		*Chroococcidiopsis* sp./Crest (Silva database) assignments		
	OTU26		*Chroococcidiopsis* sp./Crest (Silva database) assignments		
	OTU33		Uncultured *Chroococcidiopsis* sp.; FJ805849 (99)/Blast NCBI	X	X
	OTU38		Uncultured *Chroococcidiopsis* sp.; KM006906 (99)/Blast NCBI		
	OTU39		*Chroococcidiopsis* sp./Crest (Silva database) assignments		
	OTU44		*Chroococcidiopsis* sp./Crest (Silva database) assignments		
	OTU46		Uncultured *Chroococcidiopsis* sp. OTU27; MF527165.1 (99)/Blast NCBI		
	OTU47		*Chroococcidiopsis* sp.; FJ805852 (99)/Blast NCBI		
	OTU49		*Chroococcidiopsis* sp./Crest (Silva database) assignments		
	OTU50		*Chroococcidiopsis* sp./Crest (Silva database) assignments		
	OTU51		*Chroococcidiopsis* sp.; KC525099 (99)/Blast NCBI		
	OTU55		*Chroococcidiopsis* sp.; KX833848 (96)/Blast NCBI		
	OTU56		*Chroococcidiopsis* sp./Crest (Silva database) assignments		
	OTU62		*Chroococcidiopsis* sp./Crest (Silva database) assignments		
	OTU64		*Chroococcidiopsis* sp./Crest (Silva database) assignments		
	OTU66		Uncultured *Chroococcidiopsis* sp.; FJ805852 (95)/Blast NCBI		
	OTU73		Uncultured *Chroococcidiopsis* sp.; FJ805941 (96)/Blast NCBI		
	OTU78		*Chroococcidiopsis* sp./Crest (Silva database) assignments		
	OTU82		***Chroococcidiopsis* sp. ATE715** (100)	X	X
	OTU85		*Chroococcidiopsis* sp./Crest (Silva database) assignments		
	OTU67	*Synechococcus* sp.	***Synechococcus* sp. ATE729** (97.6)		
	OTU15	*Microcoleus vaginatus*	***Microcoleus vaginatus* ACT688** (99.2)	X	X
	OTU35	*Microcoleus steenstrupii*	*Microcoleus steenstrupii*; AF355379 (99)/Blast NCBI		
	OTU36		*Microcoleus steenstrupii*; AF355395 (99)/Blast NCBI		
	OTU5	Other *Microcoleus*	*Microcoleus* sp.; MF002059.1 (99.6)/Blast NCBI		
	OTU17		Uncultured *Microcoleus* sp. clone OTU_71; MF527204 (99)/Blast NCBI		
	OTU28		Uncultured *Microcoleus*; KC311890 (99)/Blast NCBI		
	OTU40		Uncultured *Microcoleus*; KC311874 (100)/Blast NCBI		
	OTU43		*Microcoleus steenstrupii*; AF355379 (96)/Blast NCBI		
	OTU48		Uncultured *Microcoleus* sp. clone OTU_28; MF527166 (100)/Blast NCBI		
	OTU65		Uncultured *Microcoleus*; KP100389 (96)/Blast NCBI		

(Continued)

TABLE 3 | Continued

	OTU	Taxonomic assignment	Best match; accession no (%ID)/source	Observed in field sample	Isolated culture
	OTU69		*Microcoleus steenstrupii*; AJ871982 (95)/Blast NCBI		
	OTU70		*Microcoleus steenstrupii*; AJ871979 (96)/Blast NCBI		
	OTU6	*Porphyrosiphon notarisii*	***Porphyrosiphon notarisii*** (99.2)	X	X
	OTU12	*Symplocastrum flechtnerae*	*Symplocastrum flechtnerae*; KF312349 (99)/Blast NCBI	X	
	OTU25	*Phormidium* sp.	*Phormidium* sp.; EU196618 (97) Blast NCBI		
	OTU53	*Wilmottia murrayi*	*Wilmottia murrayi*; AY493627 (99.2)/Blast NCBI		
	OTU76		*Wilmottia murrayi*; AY493626 (97.6)/Blast NCBI		
	OTU54	*Pseudophormidium* sp.	*Pseudophormidium* sp.; KJ939082 (100)/Blast NCBI		
	OTU22	*Chroakolemma* sp.	***Chroakolemma opaca* ACT701** (100)	X	X
	OTU30	*Oculatella* sp.	***Oculatella* sp ACT687** (99.2)	X	X
	OTU77		*Oculatella ucrainica*; MG652620 (97)/Blast NCBI		
	OTU32	*Leptolyngbya boryana*	*Leptolyngbya* boryana; EF429289 (97.6)/Blast NCBI		
	OTU71	*Leptolyngbya frigida*	*Leptolyngbya frigida*; MG641933 (100)/Blast NCBI	X	X
	OTU18	Other *Leptolyngbya*	*Leptolyngbya* sp.; AP017308 (96)/Blast NCBI		
	OTU31		***Leptolyngbya* sp. ACT691** (99.6)		X
	OTU42		*Leptolyngbya* sp./QIIME (Greengenes database) assignment		
	OTU57		*Leptolyngbya* sp./Crest (Silva database) assignments		
	OTU59		*Leptolyngbya* sp./Crest (Silva database) assignments		
	OTU63		*Leptolyngbya* sp.; HM018680 (99)/Blast NCBI		
	OTU68		*Leptolyngbya* ca. Albertano-Kovacik Green; KC463189 (99)/Blast NCBI		
	OTU72		*Leptolyngbya* sp./QIIME (Greengenes database) assignment		
	OTU95		*Leptolyngbya* sp./QIIME (Greengenes database) assignment		
	OTU96		*Leptolyngbya* sp./QIIME (Greengenes database) assignment		
	OTU61	*Trichocoleus desertorum*	*Trichocoleus desertorum*; EU586743 (99)/Blast NCBI		
	OTU86	*Phormidesmis* sp.	*Phormidesmis* sp.; KY283068 (99)/Blast NCBI		
	OTU7	Other Oscillatoriales	Oscillatoriales/Crest (Silva database) assignments		
	OTU27		Uncultured Oscillatoriales cyanobacterium OTU32 (100); MF52717170/Blast NCBI		
	OTU29		Uncultured Oscillatoriales cyanobacterium OTU40 (99); MF52717178/Blast NCBI		
	OTU94		Oscillatoriales/Crest (Silva database) assignments		
		Unassigned	OTU21, OTU34, OTU93, OTU98		
		<0.1	OTU74, OTU75, OTU79, OTU80, OTU81, OTU83, OTU84, OTU87, OTU89, OTU90, OTU91, OTU92, OTU97, OTU99, OTU100, OTU101, OTU102, OTU103, OTU104, OTU105, OTU106, OTU107, OTU108, OTU109		

Isolated strains from this study are in bold. Colors correspond to those in ***Figure 7****.*

DISCUSSION

The distribution of cyanobacterial species and their abundance is of crucial importance for understanding recent and ancient environmental dynamics. However, data about cyanobacterial species distribution are fragmented and strongly biased depending on the work carried out on a certain continent (Büdel et al., 2016). Factors influencing cyanobacterial distribution have been discussed for a long time, and climate and geography have been the main focus of attention. However, recent studies addressed the importance of soil texture on the growth of different cyanobacteria on the soil, in inoculation experiments (Rozenstein et al., 2014; Chamizo et al., 2018); whereby in areas of high fine soil grains content, a high biocrust cover was found (Belnap et al., 2014). The fact that our study area is dominated by fine-textured soils can explain why we found an extensive biocrust cover, similar to those found in previous studies (Williams et al., 2013; Belnap et al., 2014), and a high cyanobacterial diversity.

Molecular investigations through next-generation sequencing culture-independent approaches or by sequencing inserts of clone libraries have shown differences in cyanobacterial diversity depending on the geographical region. Community composition of microbial phototrophs in arid soil biocrusts of the southwestern United States showed that this region was dominated by the bundle-forming, non-heterocystous cyanobacteria *Microcoleus vaginatus* and *Microcoleus steenstrupii*; the former mostly dominated in the northern locations, and the second mostly dominated in the southern latitudes (Garcia-Pichel et al., 2013). In Artic biocrusts, cyanobacterial communities were dominated by sequences related to the form-genera *Leptolyngbya, Calothrix, Coleofasciculus, Oscillatoria,*

Stigonema, Microcoleus, and *Phormidium* (Pushkareva et al., 2015). In southern African biocrusts, in addition to typical genera found in biocrusts, such as *Microcoleus, Phormidium*, *Tolypothrix*, and *Scytonema*, sequences corresponding to the genera *Leptolyngbya, Pseudanabaena, Oscillatoria,* and *Schizothrix* were also found (Dojani et al., 2014). Analysis of the cyanobacterial diversity of western European biocrusts along a latitudinal gradient demonstrates that all the sites, except Spain, included taxa unique to the surveyed localities, but all the sites were dominated by *Leptolyngbya*, *Phormidium*, and a cyanobacterium with no matches in the databases (Williams et al., 2016). Our previous results of biocrusts from a latitudinal and climatic gradient in Spain showed a general dominance of *Microcoleus vaginatus* and *Microcoleus steenstrupii,* although differences between the locations were also found, wherein some heterocystous cyanobacteria, such as *S. hyalinum,* appeared to be dominant in developed biocrusts from the warmest and driest southwest locations (Muñoz-Martín et al., 2019).

The results from the present study revealed clear differences regarding the aforementioned studies. In addition, it is remarkable that although these localities are relatively close and belong to the same desert system (Chihuahuense), the cyanobacterial species composition and abundance are very different between the localities. The taxonomic assignment of the sequences revealed a clear dominance of the heterocystous cyanobacteria in Actopan (Hidalgo state), while in Atexcac (Puebla state), although the Nostocales were also abundant, unicellular/colonial cyanobacteria, mostly *Chroococcidiopsis* spp., represented almost a third of the cyanobacteria present. The Atexcac location harbored greater cyanobacterial diversity than Actopan; in Actopan, the 83% of the cyanobacterial phylotypes corresponded to 8 OTUs, while in Atexcac, this percentage was distributed among 42 OTUs. To the best of our knowledge, our study is the first to show such great cyanobacterial

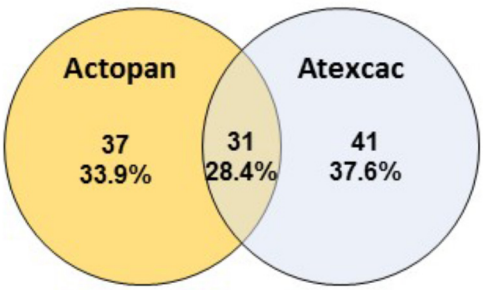

FIGURE 8 | Venn diagram showing overlap of the OTUs among the studied biocrusts.

TABLE 4 | Survival of cyanobacterial cultures over 25 days at 40°C, subsequent response of cultures to a year-long desiccation treatment at 40°C, and later survival following the addition of culture medium to the desiccated cells and maintained to room temperature.

	Survival at 40°C (days)												Survival to desiccation at 40°C	Survival after rehydration
Isolated strain	**3**	**4**	**5**	**6**	**10**	**12**	**14**	**17**	**19**	**20**	**24**	**25**		
Scytonema hyalinum ACT695	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Scytonema hyalinum ATE698	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Scytonema hyalinum ACT699	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Scytonema hyalinum ATE704	+	+	+	+	+	+	−	−	−	−	−	−	−	−
Scytonema hyalinum ACT711	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Scytonema crispum ACT685	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nostoc sp. ACT703	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Nostoc commune ACT709	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Tolypothrix distorta ATE705	+	+	+	+	+	+	+	+	−	−	−	−	−	−
Calothrix parietina ACT696	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Microcoleus vaginatus ACT688	+	+	+	+	+	+	−	−	−	−	−	−	−	−
Kamptonema sp. ACT692	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Porphyrosiphon notarisii ACT693	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Chroakolemma opaca ACT686	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oculatella sp. ACT687	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Oculatella atacamensis ATE710	+	+	+	+	+	+	+	+	+	−	−	−	−	−
Leptolyngbya frigida ACT684	+	+	+	+	+	+	+	−	−	−	−	−	−	−
Leptolyngbya sp. ACT689	+	+	+	+	+	+	+	+	+	+	+	−	−	−
Leptolyngbya sp. ACT691	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Schizothrix sp. ACT690	+	+	+	+	+	+	+	+	+	−	−	−	−	−
Chroococcidiopsis sp. ATE707	+	+	+	+	+	−	−	−	−	−	−	−	−	−
Chroococcidiopsis sp. ATE715	+	+	+	+	+	−	−	−	−	−	−	−	−	−
Synechococcus sp. ATE729	+	+	+	+	−	−	−	−	−	−	−	−	−	−

diversity in biocrusts. This high level of diversity suggests a greater ecosystem complexity than previously appreciated. Differences in the cyanobacterial populations in the studied biocrusts compared to other crusts in Mexico were also found. The microscopic analysis of biocrusts from the close Tehuacán Valley, also in the Puebla state, showed that the most common species that were present at the studied sites were *Scytonema javanicum, Microcoleus paludosus,* and *Chroococcidiopsis* sp., while *Nostoc* sp., *Schizothrix* sp., and *Aphanocapsa* sp. were found with a lower frequency, and *Gloeocapsa* sp. was the only rare species of cyanobacteria (Rivera-Aguilar et al., 2006).

Within heterocystous forms, the genera *Nostoc* and *Scytonema* were dominant at both studied locations, which may have relevant ecological implications for the nitrogen cycle and other ecosystem services. *Nostoc* spp. are considered important components of the nitrogen-fixing community in nutrient-poor soils worldwide (Dodds et al., 1995). In addition, macroscopic *Nostoc* colonies, found usually on the surface (Belnap and Lange, 2003) as well as the *Scytonema* filaments, undergo intense environmental stresses. These include high daytime temperatures during the summer, low temperatures during the night in the winter, high visible and UV radiation, and frequent hydration-dehydration cycles, which are characteristic of the conditions found at our sampling locations. The aforementioned cyanobacteria have therefore had to develop adaptive structural and physiological mechanisms to live in these places (Hu et al., 2012). One of these mechanisms for the cyanobacteria inhabiting exposed near-surface soils, is to produce photoprotective accessory pigments, such as scytonemins, mycosporine-like amino acids and carotenoids, which increase in content with solar radiation (Bowker et al., 2002), and reduce the amount of UVR damage accrued when cells are desiccated and metabolically inactive or dormant, when temperatures are suboptimal or freezing (Castenholz and Garcia-Pichel, 2012). Thus, scytonemin-producing cyanobacteria found, play an important role in protecting soil from solar radiation, shading the surrounded community and allowing colonization by other species (Garcia-Pichel and Castenholz, 1991; Singh et al., 2010).

Chroococcidiopsis spp., found in high abundance in Atexcac location, although typically considered hypolithic organisms, are also often found at the soil surface, surviving prolonged desiccation and developing sheaths containing pigments for UV protection (Dillon et al., 2002). In these extreme conditions, usual in this location, adaptation is also related to the secretion of a copious hygroscopic extracellular polymeric substance that enables cyanobacteria to cope with prolonged moisture deficit in hot deserts (Pointing, 2016).

The capacity to adapt to different environmental conditions can be distinct and depends on the attributes of cyanobacteria. Differences in climatic conditions between the studied sites were found and to investigate the possible interrelation between these climate conditions and differences in the cyanobacterial community composition, we analyzed the specific sensitivity of the isolated biocrust cultures to heat and desiccation. The results clearly showed differences between the analyzed strains which might be responsible for generating the differences in composition found. For instance, *S. hyalinum* and *N. commune*, which were able to survive to extreme conditions in the bioassays, presented higher abundances at the Actopan location, where a maximum temperature of 47°C was reached, than at Atexcac, where the maximum temperature was just 36°C. Much of the success of *Nostoc* in dryland habitats is related to its ability to remain desiccated for months, years, or even several decades (Hu et al., 2012) and to fully recover its metabolic activity within hours to days after rehydration with liquid water (Dodds et al., 1995). In fact, it has been found that *N. commune* from terrestrial habitats in China recovered after a drought period of 2 years, with reactivation of respiration, photosynthesis, and nitrogen fixation (Scherer et al., 1984). Evidence has shown that the development of specialized cells, such as akinetes (spore-like) which are cell survival stages that are known only in heterocystous cyanobacteria, leads to higher tolerance to dryness than in other stages (Hu et al., 2012). Therefore, the development of akinetes in *Nostoc* cultures can explain the observed survival after a year of desiccation at 40°C. Additionally, desiccation provides some protection to high temperature for biocrust cyanobacteria (Lan et al., 2014) which could also explain the resilience to this disturbance.

Understanding differences in the cyanobacterial diversity living in dryland areas remains essential since these biota play a critical role in ecosystem functioning. In addition to the geographic patterns of distribution discussed above, the heterogeneity of microenvironments or microclimatic gradients can also influence cyanobacterial diversity. Therefore, in our study, the nature of the cyanobacterial community structure seems to depend on these specific characteristics rather than the geographic location of the study sites. For a long time, some cyanobacteria have been reported as unique and endemic to specific sites, such as hot or polar environments (e.g., Castenholz, 1996; Taton et al., 2003). The question of endemism of cyanobacteria remains unsettled, and currently accepted theories about microbial biogeography assume that most microorganisms are cosmopolitan and ubiquitous (Fenchel and Finlay, 2004; Foissner, 2008). The paradigm "everything is everywhere, the environment selects" (Beijerinck, 1913; Becking, 1934) has been a starting point for studies of prokaryotic biodiversity and their biogeographical patterns (De Wit and Bouvier, 2006 and references therein). However, recent studies dispute the idea that 'everything is everywhere,' and the claim that 'the environment selects' implies that different contemporary environments maintain distinctive microbial assemblages (Martiny et al., 2006). Komárek (2015) found specific endemic Antarctic cyanobacterial units, inconsistent with the cosmopolitan hypothesis. Furthermore, several authors encourage the theory of cyanobacterial endemism, reporting unique distributions of some taxa. Sherwood (2004, 2007) and Sherwood et al. (2015) characterized Hawaiian freshwater and terrestrial cyanobacteria, revealing high diversity and numerous cyanobacterial species that are believed to be endemic to

the Hawaiian Islands. Kastovsky et al. (2016) described two new cyanobacterial species, presumably endemic taxa of the Chimantá Massif, Venezuela. Analysis of the cyanobacteria from hot water springs of the North-Western Himalayas harbored endemic cyanobacterial species (Singh et al., 2018). Lastly, in comparing the cyanobacteria within the biocrusts of the Arctic, Antarctic, and European Alpine sites, the discovery of a new *Oculatella* species, through the polyphasic approach, enhanced arguments for cold-assigned cyanobacterial endemism (Jung et al., 2018). The results from the present study support this view, whereby phylogenetic analysis revealed novel phylotypes not previously found with some of them present in high abundances (see, e.g., OTU 7, and those taxa corresponding mainly to *Chroococcidiopsis* spp.). Mexico contains a great diversity of topography and climate because of its complex geology, with the existence of a fair number of regions that behave as true ecological islands and peninsulas; these climatic and topographical attributes have led to the recognition of a large proportion of endemic genera, most of them related to the degree of climatic aridity (Rzedowski, 1991; Rodrigues et al., 2004) which is a trait that can be reflected in the cyanobacteria present as well. In fact, although the two sampling sites belong to the Chihuahuan Desert, they are separated by the Trans-Mexican Volcanic Belt, a forested cordillera lacking biocrusts that acts as a high elevation barrier to the dispersal of plant and animal species. Thus, our sites harbored cosmopolitan cyanobacteria, such as the typical taxa generally found in biocrusts (e.g., *N. commune, S. hyalinum, T. distorta*) but also presented novel biocrust-associated phylotypes that could be endemic. Further analysis of the biogeographical relationships and extending the sampling sites comprehensively throughout the world will determine if the current putative endemic cyanobacteria reflect the gaps in our knowledge of cyanobacterial diversity or if there is true endemism.

AUTHOR CONTRIBUTIONS

IB-A and PM designed the study. IB-A collected the biocrust samples and performed laboratory work. IB-A and GM performed the microscopic study. IB-A, MM-M, and PM performed the bioinformatics analysis of sequence data. The first draft of this manuscript was written by PM and all co-authors contributed to improve it.

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frontiers in Ecology and Evolution

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Reproduction and Dispersal of Biological Soil Crust Organisms

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Biological soil crusts (BSCs) consist of a diverse and highly integrated community of organisms that effectively colonize and collectively stabilize soil surfaces. BSCs vary in terms of soil chemistry and texture as well as the environmental parameters that combine to support unique combinations of organisms—including cyanobacteria dominated, lichen-dominated, and bryophyte-dominated crusts. The list of organismal groups that make up BSC communities in various and unique combinations include—free living, lichenized, and mycorrhizal fungi, chemoheterotrophic bacteria, cyanobacteria, diazotrophic bacteria and archaea, eukaryotic algae, and bryophytes. The various BSC organismal groups demonstrate several common characteristics including—desiccation and extreme temperature tolerance, production of various soil binding chemistries, a near exclusive dependency on asexual reproduction, a pattern of aerial dispersal over impressive distances, and a universal vulnerability to a wide range of human-related perturbations. With this publication, we provide literature-based insights as to how each organismal group contributes to the formation and maintenance of the structural and functional attributes of BSCs, how they reproduce, and how they are dispersed. We also emphasize the importance of effective application of molecular and microenvironment sampling and assessment tools in order to provide cogent and essential answers that will allow scientists and land managers to better understand and manage the biodiversity and functional relationships of soil crust communities.

Keywords: biological soil crusts (BSCs), bacteria, fungi, terrestrial algae, bryophytes, reproduction, aerial dispersal

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Biological soil crusts (BSCs) consist of various combinations of living organisms that colonize, organize, and stabilize soil surfaces against the erosive forces of wind and water. Many BSC organisms are photoautotrophic, fixing, and accumulating organic carbon (Green and Proctor, 2016) while other organismal groups fix and distribute organic nitrogen. Groups of organisms known to contribute to the formation BSC communities include cyano-, chemoheterotrophic, and diazotrophic bacteria, free-living, lichenized, and mycorrhizal fungi, terrestrial algae (including diatoms), and bryophytes (Belnap et al., 2001; Weber et al., 2016). To be recognized as a contributor to the formation and maintenance of BSC communities, an organism must be involved in the consolidation and stabilization of soil particles and aggregates, resulting in the formation of an intact BSC community. In this review of BSC organisms, we evaluate each organismal group independently, documenting how they contribute to the formation of BSCs, and how they reproduce and disperse.

BACTERIA

Prokaryotic bacteria commonly found in BSC communities include cyanobacteria, chemoheterotrophic bacteria, and free-living diazotrophic (nitrogen fixing) bacteria.

Cyanobacteria

Cyanobacteria are photoautotrophic prokaryotes (Whitton and Potts, 2000). Traditionally, cyanobacteria were classified with the eukaryotic "algae" based on the presence of chlorophyll a and the production of molecular oxygen. However, given the absence of membrane bound subcellular structures (e.g., nuclei, mitochondria, etc.) and the occurrence of prokaryotic type ribosomes, the cyanobacteria are now classified as bacteria. Cyanobacteria also produce a unique cell wall chemistry containing peptidoglycans rather than cellulose. Thallus-types range from unicellular to multicellular filaments, sheets, or globular thalli (Schirrmeister et al., 2011; Herrero et al., 2016). Numerous genera of BSC cyanobacteria have been reported—spanning five orders, Chroococcales, Nostocales, Oscillatoriales, Pleurocapsales, and Synechococcales. Cyanobacteria are recognized as one of the most important and abundant photoautotrophs in many arid land BSC communities (Colesie et al., 2016)—occurring at or just below the soil surface (Hu et al., 2003). Through photosynthesis, cyanobacteria contribute significant fixed carbon to the BSC community. Many species, including many of the filamentous and globular forms, also produce heterocysts, specialized nitrogen fixing structures (Fay, 1992; Bergman et al., 1997; Kumar et al., 2010). Nitrogen fixing cyanobacteria, both as free living and symbiotic forms, also contribute organic nitrogen to the general soil crust community (Bergman et al., 1997). Filamentous species such as *Microcoleus* are capable of binding together into rope-like structures that allows them to colonize physically unstable sedimentary environments (Garcia-Pichel and Wojciechowski, 2009). Some filamentous species also secrete exopolysaccharides that effectively aggregate soil particles and thus contribute to BSC structure and stability (Rossi and De Philippis, 2015). Terrestrial cyanobacteria are poikilohydric—tolerating severe desiccation as well as high levels of UV light (Karsten and Holzinger, 2014). The UV filtering capacity of terrestrial cyanobacteria is related to the presence of light screening compounds found in their cells and sheath material, often resulting in the characteristic dark color typical of many cyanobacteria-dominated BSC communities (Scherer et al., 1988; Rosentreter et al., 2007; Rastogi and Incharoensakdi, 2014). During winter months, the authors have anecdotally observed that the dark surface of BSC communities in the Great Basin tends to absorb winter sunlight, causing snow and ice to melt providing the BSC community with liquid water that in turn potentially supports brief but important periods of metabolic activity.

Reproduction in the cyanobacteria is strictly asexual (Mur et al., 1999) and may be accomplished by budding (Waterbury and Stanier, 1977), non-specialized thallus fragments (Meeks and Elhai, 2002), binary fission (Kunkel, 1984), or the formation of specialized asexual structures (Somon, 1977) including akinetes (Kaplan-Levy et al., 2010). Hormogonia—specialized thallus fragments commonly produced by some filamentous cyanobacteria (Campbell and Meeks, 1989; Meeks and Elhai, 2002) also function as effective asexual propagules. Cyanobacteria act as pioneer species while also commonly occurring in more mature BSC communities as both free living and symbiotic taxa.

Cyanobacteria have been found in BSC communities on all continents, and upwards of 50 taxa have been identified from regional samples of BSC communities (Rippin et al., 2018). Many terrestrial cyanobacteria have also been collected from the atmosphere (Sharma and Singh, 2010; Genitsaris et al., 2011) and are among the most numerous airborne microorganisms reported from aerial samples (Sharma and Singh, 2010; Després et al., 2012). In terrestrial environments, filamentous cyanobacteria are able to glide along thin layers of water coating soil particles when the soil is moist (Castenholz, 1982; Hoiczyk, 2000). However, in terrestrial environments, local dispersal distances are extremely limited (cm scale) compared to dispersal through the air (km or intercontinental scale). In Antarctica, cyanobacterial communities in close proximity to each other had a low degree of similarity with each other, indicating the probability of longer-distance aerial transport (Namsaraev et al., 2010).

Chemoheterotrophic Bacteria and Free-Living Diazotrophic Bacteria

Chemoheterotrophic bacteria and free-living diazotrophic bacteria commonly integrate and closely interact with other BSC organismal groups. They are, however, one of the least studied and understood constituents of BSCs. These bacteria reside in a nutrient-rich zone where cyanobacteria and other biocrust constituents create a "cyanosphere" (Couradeau et al., 2019) with relatively enhanced organic carbon, nitrogen, and water availability. Large numbers of soil heterotrophic bacteria are positively correlated with BSC stability (Andrade et al., 1998; Makhalanyane et al., 2015; Nunes da Rocha et al., 2015). Many heterotrophic bacteria are filamentous and/or produce exopolysaccharides (Crania et al., 2019), characteristics that contribute to the formation and stabilization of BSCs. The occurrence of these BSC bacteria are ultimately a product of multiple environmental filters (e.g., climate, soil type, and disturbance regimes Eldridge and Delgado-Baquerizo, 2019. Each crust type (cyanobacteria dominated, lichen-dominated, and bryophyte-dominated) exerts control over the bacterial phyla and families present (Maier et al., 2018). To begin to identify the higher-taxonomical convergence among BSC bacteria, other than cyanobacteria, we examined the patterns of abundant bacterial families in the three crust types (**Table 1**). Of the 24 dominant heterotrophic bacterial orders/families found to occur in BSC communities over eleven studies, nine were cosmopolitan taxa [i.e., present in all three biocrust types, Acidobacteriaceae (Acidobacteria), Rubrobacteraceae (Actinobacteria), Shingomonadaceae (Alphaproteobacteria), Bradyrhizobiaceae (Alphaproteobacteria), Rhodobacterales

(Alphaproteobacteria) Chitinophagaceae (Bacteroidetes), Cytophagaceae (Bacteroidetes), Oxalobacteraceae (Betaproteobacteria), Chthoniobacteraceae (Verrucomicrobia)], while five were unique to either cyanobacteria-dominated [i.e., Solirubrobacterales (Actinobacteria), Burkholderiales (Betaproteobacteria), Trueperaceae (Deinicoccus-Thermus) or lichen-dominated crusts [i.e., Armatiomonadaceae (Armatimonadetes), Cystobacteraceae (Deltaproteobacteria)]. The Alphaproteobacteria contained the greatest number of families at five, followed by the Actinobacteria that housed four families or orders. The most commonly found taxon in cyanobacteria-dominated crusts was the Rubrobacteraceae (Actinobacteria), and Shingomonadaceae (Alphaproteobacteria) in lichen- and moss-dominated crusts. These patterns may serve as a starting point for a conversation to find novel community patterns among biocrust copiotrophs. In all BSCs, regardless of the type, heterotrophic bacteria exert significant influence over soil biogeochemistry and structure by regulating carbon and nitrogen cycling (Schimel and Schaeffer, 2012; Nelson et al., 2016). In addition, the production of exopolymeric compounds by many heterotrophic bacteria contributes to soil aggregation that has a direct influence on water infiltration and soil structure (Costa et al., 2018). The presence of free-living diazotrophs (N-fixing bacteria and archaea) within the cyanosphere (Couradeau et al., 2019) adds essential available nitrogen to predominantly nitrogen-limited desert soils. In *Microcoleus vaginatus*-dominated crusts, Couradeau et al. (2019) identified multiple heterotrophic diazotrophs associated with several taxa from the Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria groups. Furthermore, in developing cyanobacteria-dominated BSCs several of the same diazotrophs were identified by Pepe-Ranney et al. (2016) using 15N-DNA SIP methods.

Biological soil crust bacteria, like other soil bacteria, reproduce rapidly by binary fission when conditions are favorable. Otherwise, they enter a reversible state of reduced metabolic activity or dormancy. Dormancy establishes reservoirs of inactive individuals that resume metabolic and reproductive activity when appropriate environmental conditions return (Lennon and Jones, 2011; Joergensen and Wichern, 2018). Potentially, as much as 90% of the BSC microbial community is inactive with more than 50% of all bacterial taxa dormant during periods of stress (Alvarez et al., 1998; Lennon and Jones, 2011; Aanderud et al., 2015). Invariably, the single most limiting resource contributing to the onset of dormancy in the BSC community is soil moisture. For example, using H218O RNA-stable isotope probes in a cyanobacteria-dominated BSC community in Israel's Negev Desert, the rewetting of dry crusts caused bacteria to grow, resulting in the development of a distinctly different bacterial community with the previously dominant Actinobacteria population significantly reduced (Angel and Conrad, 2013). BSCs host a diverse bacterial community whose members grow, interact, and alter community composition depending on environmental conditions and activities that may impact BSC structure and health.

The primary dispersal mechanism for heterotrophic bacteria and diazotrophs within the BSC community is bioaerosols. Although BSCs generally stabilize desert soils against wind erosion, dust entrainment is the primary pathway for the movement of BSC bacteria from arid and semi-arid BSC communities. The various biological components of BSCs differ in their contributions and role in reducing soil erosion (Belnap and Gardner, 1993; Mazor et al., 1996; Bowker et al., 2008; Tisdall et al., 2012). Notwithstanding all the erosion benefits provided by BSCs, deserts remain a primary source of dust globally with desert winds aerosolizing several billion tons of soil-derived dust each year (Kellogg and Griffin, 2006). Local dispersal of bacteria occurs through the soil matrix but is extremely limited (at a cm scale) compared to dispersal through the air (intercontinental, at a km scale) (Choudoir et al., 2018). Dust from deserts may travel across continents providing an opportunity for BSC organismal groups to disperse across long distances. For example, dusty snow on Mont Blanc in the alps on the French and Italian border contained soil bacteria deposited by four Saharan desert dust storms over a 3-year period (Chuvochina et al., 2011). Similarly, dust from deserts in Chad were deposited in the Cape Verde Islands with many of the dust-borne bacterial families identical to families commonly reported for BSC communities (Favet et al., 2013). Dust contains an immense diversity of bacteria (Choudoir et al., 2018; Dastrup et al., 2018) and retains much of the diversity profile typical of soil surfaces (Boose et al., 2016; Weil et al., 2017; Dastrup et al., 2018). Dust also acts as a source of wind-borne propagules with dust particles settling in response to gravity or acting as ice nucleators enhancing snowfall (Christner et al., 2008). Dust entrainment from deserts is also enhanced by anthropogenic disturbance of soil surfaces (Belnap and Gillette, 1998). Disturbed BSC communities have been shown to serve as a source of inoculants (Warren et al., 2019), further enhancing dispersal bridges between crust communities or into open habitats—suitable for biocrust development.

FUNGI

Three categories of fungi are reported for BSC communities including free-living fungi, lichenized fungi, and mycorrhizal fungi. All three groups of terrestrial fungi play important ecological roles in the establishment and maintenance of BSC communities.

Free-Living Fungi

Free-living fungi are eukaryotic heterotrophs that acquire energy and matter from other organisms (living or dead). They produce a wide range of enzymes that degrade chitin, keratin, cellulose, and lignin (Alexopoulos et al., 1996; Kendrick, 2017), substrates that are not readily degraded by other organisms. Fungi have the unique ability to digest/consume organic substrates while also releasing compounds that are often appropriated by other organisms. Moreover, fungi are also able to survive extreme environments such as temperature extremes, desiccation, pH extremes, etc. (Kendrick, 2017). Fungi in general are recognized for their role as decomposers, and their ability to withstand environmental extremes. They are now often included in the suite of microorganisms that comprise BSCs, primarily because of their contributions to the process of consolidating and

TABLE 1 | Abundant chemoheterotrophic bacterial families/orders in cyanobacteria-dominated, lichen-dominated, and/or bryophyte-dominated biocrusts based on eleven studies.

Phylum or Class	Orders/Family	Cyanobacteria-dominated	Lichen-dominated	Bryophyte-dominated
ACIDOBACTERIA				
	Acidobacteriaceae	Maier et al., 2018	Maier et al., 2018; Aanderud et al., in review	Maier et al., 2018
ACTINOBACTERIA				
	Actinomycetales Actinomycetaceae	Gundlapally and Garcia-Pichel, 2006; Angel and Conrad, 2013	Maier et al., 2014	
	Nocardioidaceae		Maier et al., 2018	Aanderud et al., in review
	Rubrobacteraceae	Nagy et al., 2005; Gundlapally and Garcia-Pichel, 2006; Angel and Conrad, 2013; Maier et al., 2018	Kuske et al., 2012; Maier et al., 2018; Aanderud et al., in review	Maier et al., 2018
	Solirubrobacterales	Angel and Conrad, 2013		
ALPHAPROTEOBACTERIA				
	Shingomonadaceae	Maier et al., 2018	Kuske et al., 2012; Steven et al., 2013; Maier et al., 2014, 2018; Aanderud et al., in review	Moquin et al., 2012; Navarro-Noya et al., 2014; Maier et al., 2018; Aanderud et al., in review
	Bradyrhizobiaceae	Maier et al., 2018	Maier et al., 2018	Navarro-Noya et al., 2014; Maier et al., 2018
	Rhizobiales Methylobacteriaceae	Angel and Conrad, 2013; Steven et al., 2014		Aanderud et al., in review
	Rhodobacterales Rhodobiaceae	Angel and Conrad, 2013; Maier et al., 2018	Maier et al., 2018	Maier et al., 2018
	Rhodospirillales Acetobacteraceae	Angel and Conrad, 2013		Aanderud et al., in review
	Rubrobacteraceae	Angel and Conrad, 2013		
ARMATIMONADETES				
	Armatiomonadaceae		Maier et al., 2018	
BACTEROIDETES				
	Chitinophagaceae	Maier et al., 2018	Kuske et al., 2012; Maier et al., 2018	Maier et al., 2018
	Cytophagaceae	Maier et al., 2018	Maier et al., 2018	Maier et al., 2018
	Sphingobacteriales	Angel and Conrad, 2013	Maier et al., 2014	
BETAPROTEOBACTERIA				
	Burkholderiales	Angel and Conrad, 2013		
	Oxalobacteraceae	Nagy et al., 2005	Maier et al., 2018	Moquin et al., 2012
DEINICOCCUS-THERMUS				
	Trueperaceae	Maier et al., 2018		
DELTAPROTEOBACTERIA				
	Cystobacteraceae		Steven et al., 2013	
VERRUCOMICROBIA				
	Chthoniobacteraceae	Maier et al., 2018	Maier et al., 2018	Maier et al., 2018

Abundance is based on taxa possessing >1% of the community relative recovery from next-generation sequencing or a high percent of sequences from cloning efforts. Taxa from polar biocrusts are excluded due to polar crusts often being phylogenetically distinct. Studies investigating lichen- and moss-dominated biocrusts contained cyanobacteria with some moss-dominated biocrusts containing low levels of lichen cover and vice-vs. for lichen-dominated crusts.

stabilizing soil particles and aggregates (Caesar-TonThat and Cochran, 2001; States et al., 2001). Free-living fungi are found in greater abundance in well-developed BSCs than uncrusted neighboring soils (Maier et al., 2016). Although free-living fungi have been recognized as an important component of the BSC micro-community, research on free-living fungal communities in biocrusts has been limited and generally descriptive in nature (Bates and Garcia-Pichel, 2009; Bates et al., 2012; Steven et al., 2015). Ascomycota represents the predominant fungal phylum in BSCs (Maier et al., 2016). However, research has shown that the composition of BSC fungal communities differs based on soil types and geographical location (Reininger et al., 2015). In general, fungi produce vegetative filaments (hyphae) which bind soil particles together and help to consolidate the surface of BSC communities (Tisdall, 1991; Degens et al., 1996). More specifically, many of the major BSC free-living fungi in the Ascomycota are classified in the Dothideomycetes class of the Pleosporales order. Members of this group of fungi are called dark-septate endophytic fungi, due to the dark pigments commonly found in their cell walls. These pigments are usually melanin and mycosporine-like amino acids which provide protection in environments of severe desiccation

and high levels of UV light (Oren and Gunde-Cimerman, 2007; Gostinčar et al., 2009). There are two types of melanin produced by melanized fungi: 1,8-DHN melanin and L-Dopa melanin (Henson et al., 1999). Dark-septate fungi have also been suggested as contributors to nutrient links between desert vascular plants and the BSC community (Maier et al., 2016). As a result, free-living fungi have been proposed as an important component in the fungal loop hypothesis that suggests that nutrient exchange and the vascular plant-BSC connection may be mediated by fungi (Collins et al., 2008; Green et al., 2008).

While almost all free-living fungi are capable of sexual reproduction, sex is costly (Aanen and Hoekstra, 2007), and most fungi generally reproduce using a variety of asexual mechanisms (Nieuwenhuis and James, 2016; Ojeda-López et al., 2018). Asexual reproduction may be accomplished by fission or budding of somatic cells, fragmentation of mycelial strands or hyphae, or production of asexual spores. All such processes result in haploid individuals. Because sexual reproduction is possible yet infrequent, outcrossing is, likewise, rare to infrequent (Nieuwenhuis and James, 2016; Ojeda-López et al., 2018). Indeed, sexual reproduction is infrequent in many fungi, yet self-fertility is relatively common (Lee et al., 2010). In BSCs, free-living fungi are generally classified based on their asexual reproductive structures and confirmed more specifically by limited observations of sexual fruiting bodies (Maier et al., 2016). BSCs are dominated by Dothideomycetes fungi (Steven et al., 2015) which are most often found in their anamorph (asexual) state and are recognized as filamentous molds when cultured in the laboratory. They reproduce asexually by producing non-motile conidia type spores that can be dispersed by wind or insects. Black yeasts are a second common morphotype encountered when culturing fungi from BSCs and encompasses two distinct fungal evolutionary lineages, Dothideomycetes and Chaetothyriales (Eurotiomycetes). The black yeasts are characterized by thick, melanized cell walls, and exopolysaccharide production that support a variety of extremophile properties that allow them to endure high UV radiation and extreme desiccation, while also contributing to the consolidation of soil particles. The black yeasts have only been documented to reproduce asexually through the production of conidia type spores and unicellular budding. Some species of black yeasts produce fission or budding divisions when grown in submerged environments while others are capable of producing conidiophores when growing in low moisture environments (Seyedmousavi et al., 2014).

Some free-living fungi produce specialized structures that forcibly eject thousands of spores into the air (Roper et al., 2010; https://www.anbg.gov.au/fungi/dispersal.html). Ascomycota are the dominant group of fungi in BSCs, and are also the dominant fungi found in aerobiota (Frölich-Nowoisky et al., 2016). Generally, fungal species, which rely on windblown dispersal, produce aerodynamically shaped spores that can be transported thousands of kilometers through the atmosphere (Golan and Pringle, 2017). Abundant and diverse numbers of fungal spores have been collected from the atmosphere above Antarctica (Marshall, 1997), Australia (Mitakakis and Guest, 2001), Brazil (Womack et al., 2015), Chile (Ibañez et al., 2001), China (Fang et al., 2005), India (Priyamvada et al., 2017), Iran (Shams-Ghahfarokhi et al., 2014), Italy (Sandrone, 2014), Kuwait (Halwagy, 1994), Mexico (Rosas et al., 1990), Nigeria (Ezike et al., 2016), Poland (Stępalska and Wolek, 2005), Spain (Sabariego et al., 2007), and the United States (Dupont et al., 1967), among others. The quantity and diversity of airborne fungal spores is seasonally variable (Reineria Diaz et al., 1998; Kasprzyk and Worek, 2006). Their function is dispersal followed by growth and development of vegetative structures when essential resources and ideal environmental conditions are available (Kendrick, 2017). Due to the arbitrary nature of wind dispersal, spores may not encounter conditions suitable for germination and viability. To increase their chances of reproductive success, many fungi produce and release an "over-abundance" of spores throughout the year to ensure that a sustainable number encounter a suitable environment with essential resources to accommodate germination and establishment (Kendrick, 2017). The regional production of spores is dependent on the availability of water and local spore trapping show a cyclical pattern dependent on moisture patterns. Global dispersal of fungal spores is facilitated by dust-borne transport that in turn supports proliferation processes (Shinn et al., 2003).

Lichenized Fungi

Lichenized fungi include more than 19,000 species (Lücking et al., 2016, 2017). Lichens are composite systems involving complicated symbiotic interactions between heterotrophic fungi primarily of the phylum Ascomycota or less frequently of the phylum Basidiomycota, and a green alga and/or a cyanobacterium (Brodo et al., 2001). The fungus, or mycobiont, provides structure and a favorable living environment in the form of a thallus or vegetative plant-like body undifferentiated into true stems, roots, and leaves, and lacking a vascular system. The green alga and/or cyanobacterium photobionts provide a carbon-based food source for themselves and the mycobiont through photosynthesis. In those cases where the photobiont is a cyanobacterium, it also fixes atmospheric nitrogen. Lichen scientific names are based strictly on the fungal symbiont, which can cause some confusion when trying to determine if a BSC fungus is lichenized or not—unless an intact lichen thallus is observed or collected and identified by a trained lichenologist. Lichens are broadly classified based on their thallus morphology as being crustose, squamulose, foliose, or fruticose. Crustose lichens are crust-like. They are slow-growing and adhere tightly to the substrate or, in some cases, are physically integrated into the surface of the substrate (Armstrong and Bradwell, 2010). Squamulose lichens are composed of tightly clustered or overlapping scale-like structures (squamules) with no or only a poorly developed lower surface. However, squamules of some squamulose species form rhizoidal hyphae on their lower surface that effectively anchor the squamules to the soil surface. Squamulose lichens constitute one of the most common growth forms associated with BSCs (St. Clair et al., 1993). Foliose lichens are leaf-like, with distinctive upper and lower surfaces and are generally loosely attached to the substrate by root-like structures called rhizines (Brodo et al., 2001). Fruticose lichens are highly branched and are either upright and shrubby or pendant with a

single point of attachment (Baron, 1999). BSC lichen dominated communities tend to be more common in undisturbed locations with well-developed vertical BSC structure (Rosentreter et al., 2007). Some BSC lichens, mostly foliose forms referred to as "vagrant lichens" (e.g., the lichen genera *Xanthoparmelia* and *Rhizoplaca*), occur unattached on the soil surface (Rosentreter, 1993). Generally, some of the more common BSC lichen genera include *Psora, Placidium, Xanthocarpia, Lecanora, Circinaria,* and *Acarospora.* Lichens have a broad distribution, occurring on a variety of substrates and occupying habitats ranging from the humid tropics to hyper-arid zones, and from the frigid tundra to hot deserts. When dry, they suspend all metabolic activity and are able to tolerate extremely high temperatures for extended periods of time. Lichens occur on a variety of substrates, including soil, rocks, monuments, statues, roofs, walls, fences, bark, and decorticated wood (St. Clair and Seaward, 2004). Lichens contribute to the weathering of stone substrates by producing weak organic acids (Chen et al., 2000; Souza-Egipsy et al., 2004; Leger and Forister, 2009). Lichens are desiccation-tolerant allowing them to inhabit very harsh environments and experience drought conditions for extended periods (Nash et al., 1990; Kranner et al., 2008).

Some lichens reproduce asexually using unspecialized thallus fragments (Armstrong, 2017; Almeida Pereira et al., 2018) or through the formation of small-specialized asexual structures containing cells of both the mycobiont and the photobiont. These specialized asexual structures include soredia, isidia, and lobules (Ott et al., 1993). Soredia are small, spherical bundles of algal cells wrapped in a mesh of fungal hyphae. Soredia frequently occur as powdery patches (soralia), which erupt through the upper surface (cortex) of some lichens. Isidia are specialized thallus fragments of various shapes and configurations attached to the upper surfaces of lichens. Like soredia, isidia contain both algal cells and fungal hyphae; however, unlike soredia the surfaces of isidia are encased in a layer of cortical cells. Lobules are small, flattened, variously shaped structures that generally occur along the edges of the thallus of some lichens. These specialized asexual regenerative structures are easily detached from the thallus surface by wind, rain, or other physical disturbance (Blackwell et al., 1996) and may subsequently be dispersed to a new suitable substrate where they become attached and form a new lichen thallus. Lichens commonly reproduce sexually as well; however, sexual reproduction in lichens is limited to the fungal partner, which produces sexual spores called ascospores within fruiting bodies called ascocarps. The photobiont (alga or cyanobacterium) is also capable of sexual reproduction, but, unlike the fungus, not while engaged in the lichen symbiosis. The primary limitation to sexual reproduction in lichens is the obstacle of the germinating ascospore encountering a viable photobiont partner in the process of attempting to reconstitute a new lichen thallus (Bowler and Rundel, 1975). However, the fact that many broadly distributed lichen species reproduce only sexually through the production of ascospores suggests that the likelihood of an ascospore germinating and surviving long enough to encounter an appropriate photobiont happens with impressive frequency. Though little is known about the dispersal of lichen gametes, the gametes of the lichen-forming fungus, *Lobaria pulmonaria*, are known to have an aerial dispersal range of several hundred meters to kilometers from mature individuals. On the other hand, clonal propagules (i.e., soredia and isidia) disperse over a distance of tens of meters while spores may be dispersed for several kilometers (Ronnås et al., 2017). However, joint dispersal of the mycobiont and photobiont does not necessarily imply that the two will be rejoined (Wornik and Grube, 2010). Indeed, they may switch symbiotic partners (Ertz et al., 2018).

Lichen thalli, are readily dispersed into adjacent and expanded habitats as both sexual and asexual propagules. Dispersal of either non-specialized or specialized thallus fragments over significant distances is accomplished by wind, water, or as attached to animal feathers, fur, or feet (Bailey, 1966). Researchers in northeastern Germany, a region not known for high winds, found that the dispersal distance of lichen fragments was negligible, and was measured in centimeters (Heinken, 1999). In contrast, areas where high surface winds are more common, lichen thallus fragments, detached reproductive structures (soredia, isidia, lobules), and spores can be lifted into the atmosphere and transported very long distances (Després et al., 2012). For example, lichen soredia have been collected at high altitudes in southwest Spain (Tormo et al., 2001) and Antarctica (Marshall, 1996). Airborne asexual lichen fragments have been collected from rooftops throughout the continental United States (Tripp et al., 2016). On an ocean voyage from the Polish Antarctic Station on King George Island by way of South Shetland Island to Gdynia, Poland, scientists intended to collect pollen grains daily on blotter paper. In addition to pollen, the scientists commonly found fungal spores, lichen thallus fragments, isidia, and soredia in abundance in all samples, indicating widespread distribution of airborne lichen propagules (Harmata and Olech, 1991). The abundance and distribution of fungal spores vary by species and climate (Marshall, 1997). The lichen genus *Ramalina* is found on the main island of New Zealand, as well as the outlying islands of New Zealand (Bannister and Blanchon, 2003), suggesting long-distance dispersal. The foliose lichen genus *Xanthoparmelia*, purportedly originated in South Africa, but is now present in South and North America as well as Australia (Amo de Paz et al., 2012).

Mycorrhizal Fungi

Mycorrhizal fungi are obligate plant root symbionts that provide their host plants with increased water and nutrient absorption capacity while the plant provides the fungus with carbohydrates from photosynthesis (Berruti et al., 2015). The roots of more than 80% of terrestrial plant species are obligatorily associated with mycorrizal fungi (Pirozynski, 1981; Smith and Read, 2008). In a mycorrhizal association, the fungus colonizes the host plant's roots, either intracellularly as in endomycorrhizal fungi or extracellularly as in ectomycorrhizal fungi. In endomycorrhizal fungi, the fungal hyphae penetrate the cortical layer of cells below the epidermis of the plant root. Some endomycorrhizal fungi form an intercellular multi-branched hyphal structure with slightly swollen chambers to store nutrients. Such a structure resembles a tree. Hence, such endomycorrhizal fungi

are often referred to as vesicular mycorrhizae or vesicular-arbuscular mycorrhizae, or simply VM or VAM for short. All vesicular or vesicular-arbuscular mycorrizae are formed by members of the Glomeromycota phylum of the kingdom fungi (Kehri et al., 2018). There is a trend toward host-specificity among mycorrhizal fungi (Torrecillas et al., 2012) such that mycorrhizal fugal diversity can be used as an index of plant biodiversity in similar ecosystems (van der Heijden et al., 1998). Soils with vesicular arbuscular mycorrhizal fungi have significantly more stable aggregates than soils without (Andrade et al., 1995). The mycelia of mycorrhizal fungi can extend several centimeters beyond the plant roots, forming a mycelial mesh that binds soil particles (Miller and Jastrow, 1992; Soka and Ritchie, 2014). The mycelia also produce a glue-like glycoprotein, glomalin, that functions as an adhesive that binds soil particles together thus reducing soil erosion (Gianinazzi et al., 2010; Singh, 2012; Gomathy et al., 2018; Prasad et al., 2018), while enhancing the structural integrity of the BSC community (Chaudhary et al., 2009).

Sexual reproduction in Glomeromycota is unknown (Pawlowska, 2005). Hence, reliance on asexual modes of reproduction is essential. Typically, mycorrhizal fungi reproduce by asexual spores (Camargo-Ricalde, 2002; Marleau et al., 2011) or by hyphal fragments or plant roots containing mycorrhizal hyphae (Berruti et al., 2014).

Dispersal of mycorrhizal fungi is facilitated by the activity of burrowing insects, rodents as well as birds that disperse spores or hyphal fragments for short distances or expose them to the erosive forces of water and wind (Camargo-Ricalde, 2002) that can move them much farther. Recent research has documented large numbers of airborne mycorrhizal spores in a variety of different biomes and ecoregions in North America where they were previously unknown (Egan et al., 2014). Spores were most frequent in the air above more arid ecosystems, suggesting that they are more likely dispersed aerially from those systems. Efforts to detect and identify airborne mycorrhizal propagules is often difficult and tends to under-report some fungal taxa. The use of molecular approaches such as DNA metabarcoding, however, can uncover fungal diversity missed by traditional morphological techniques including microscopy (Pashley et al., 2012; Chen et al., 2018), thus providing a more comprehensive estimate of fungal diversity in aerial samples (Banchi et al., 2018).

TERRESTRIAL ALGAE

Terrestrial eukaryotic algae of BSC communities include green algae, diatoms, xanthophytes, and eustigmatophytes. Like cyanobacteria, eukaryotic algae are photoautotrophic, using energy from sunlight to synthesize energy-rich organic compounds. Unlike cyanobacteria, they possess well-defined, membrane-bound, subcellular structures (e.g., nuclei, mitochondria, etc.) and may reproduce sexually. Although eukaryotic algae are usually aquatic, many species inhabit the soil, and participate in the formation of BSCs. Here we focus specifically on two groups of eukaryotic algae that are known to contribute to the structure of BSCs, namely, the green algae and diatoms.

Green Algae

Green algae of BSCs include free living and lichenized species, and most are unicellular, colonial, or filamentous in morphology. Several classes of green algae (Chlorophyceae, Trebouxiophyceae, Ulvophyceae, Klebsormidiophyceae, and Zygnematophyceae) including dozens of genera are found in BSC communities. In many cases, species of BSC green algae are closely related to aquatic sister species found in freshwater habitats, suggesting that the evolutionary transition to BSC habitats has happened many times in the green algae (Lewis and Lewis, 2005; Fučíková et al., 2014). As a group, green algal cell walls are highly diverse and include cellulose as well as other polysaccharides, and glycoproteins (Domozych and Domozych, 2014). Under certain conditions, filamentous green algae (e.g., *Klebsormidium*) may achieve high biomass and contribute to the consolidation of soil particles in BSCs due to their sticky polysaccharide-containing cell sheaths and exudates (Hoppert et al., 2004; Warren, 2014; Büdel et al., 2016). Other green algae may have lower biomass, but they also may produce sheath material that directly adheres to cyanobacteria, fungi, and other green algae (Hoppert et al., 2004). Hu et al. (2003) characterized the vertical profile of algae in a BSC "horizon," intricately mapping the physical locations of green algae and other species. Green algae typically are distributed just below the surface of desert crusts, but they are known to form thick mats at the soil surface in other types of BSC communities (Hu et al., 2003; Hoppert et al., 2004). Some green algal taxa also live symbiotically as lichen photobionts and are typically distributed just below the surface of lichen thalli, their cells protected by fungal sunscreens (Nguyen et al., 2013).

Among BSC green algae, propagules include zygotes, asexual spores, or filament fragments. In nearly all cases, the green algae of BSC are small, unicellular, with dominant haploid vegetative stages. They can reproduce sexually, or asexually by fragmentation of filaments or simple cell division, by autospores (non-motile cells formed inside the mother cell wall), and through zoospores (flagellated cells). In aquatic relatives of BSC green algae, the diploid stage (zygote) is considered the stage that persists under conditions not supporting active growth (e.g., lack of water, freezing temperatures). For example, in temperate regions, the zygotes of pond algae persist in sediments over winter, and in the spring they undergo meiosis directly to form haploid vegetative cells. Likewise, if zygotes were formed by BSC green algae, they also could potentially serve as resistant aerial propagules. Motile cells have been documented in BSC species (Flechtner et al., 2013) but it is not clear if these represent gametes or zoospores because these stages often are morphologically similar. Sexual reproduction is difficult to demonstrate in unicellular terrestrial green algae unless the fusion of motile gametes is observed directly or zygotes are observed. Sex is difficult to induce even in the laboratory, and generally is not tested in cultured algae. It is also impossible to detect directly in field-collected material. That said, sexual reproduction is thought to be possible in these species, even if rare. Support for this is indirect, coming from surveys of genomic and transcriptomic

data for meiosis genes in diverse eukaryotes, which are present in many trebouxiophycean genera, including terrestrial taxa (Fučíková et al., 2015). However, compared to algae living in aquatic habitats the opportunities for sexual reproduction in BSC taxa is greatly constrained because green algae require liquid water for their swimming gametes and because green algae in BSCs often are not present in high densities.

Green algae of BSCs do not necessarily need specialized spores or zygotes to disperse. Reproductive propagules of unicellular and multicellular eukaryotic algae are mostly dispersed aerially (Brown et al., 1964; Broady and Smith, 1994; Tesson et al., 2016). Genitsaris et al. (2011) documented 353 morphological taxa that have been reported in the literature related to aerobiology, with more reported regularly. The fact that new, cryptic species are being discovered and described (Cardon et al., 2008) suggests that additional species will be added to the list of aerobionts. Many algae occur in both the Arctic and Antarctic polar regions demonstrating a significant capacity for global long-distance dispersal (Jungblut et al., 2012). In addition to their occurrence in BSCs, airborne algae may also be deposited and survive on a variety of other substrates, including tree bark (Kharkongor and Ramanujam, 2014), glaciers and snow fields (Takeuchi, 2001; Kvíderova, 2012), polar fellfields (Marshall and Chalmers, 1997), and exterior building walls (Nakajima et al., 2015).In fact, the ability of vegetative cells to survive without water can be immediate. Hydrated, actively growing cells from diverse genera of green algae have been shown to survive rapid desiccation (Gray et al., 2007; Cardon et al., 2008) indicating the presence of mechanisms that prepare even actively growing cells for desiccation. In other species, the ability to survive is a function of the age of vegetative cells. For example, compared to young cells of the alpine taxon *Zygnema* that are tolerant to exposure to high UV-B stress (Holzinger et al., 2018) and Herburger et al. (2015) demonstrated that older vegetative cells (akinetes) are more tolerant to desiccation. Such stages likely are important to the ability of some species to survive long-range dispersal. To be part of the aerial flora, and in order to successfully colonize BSC habitats after dispersal, algae need to produce structures that can be lifted and moved in the air, and they need traits that enhance survival under drying conditions (Sharma and Singh, 2010). These algae exhibit the hallmarks of poikilohydry, by equilibrating to ambient humidity and having rapid responses upon water gain [reviewed in Green and Proctor (2016)]. Green algae, like cyanobacteria, and other photosynthetic species must also be able to survive in high light and possibly high UV habitats (Karsten and Holzinger, 2014), allowing them to survive in harsh, high elevation terrestrial habitats and during transport, deposition, and settlement while airborne (Tesson et al., 2016). Protective compounds including carotenoids, mycosporine-like amino acids, and a variety of phenolics are known in green algae (Kitzing et al., 2014; Holzinger et al., 2018). Diverse airborne green (and other) algae were noted by early researchers including van Overeem (1937) and Brown et al. (1964), with a recent review by Tesson et al. (2016). Given that BSC green algae are so diverse, and that different reproductive stages can survive dispersal, it is reasonable to assume that the underlying mechanisms that allow these species to live in habitats supporting BSCs may also vary (Holzinger and Karsten, 2013). While airborne, microalgae are tolerant to freezing temperatures and desiccation and are important ice nucleators contributing to the formation of ice and snow (Tesson and Šanti-Temkiv, 2018).

Diatoms

Diatoms, the second group of eukaryotic algae included in this section are commonly found in BSC communities (Ettl and Gärtner, 1995; Büdel et al., 2016). Diatoms are species diverse and best known from aquatic habitats, often being in high abundance in both marine and freshwater communities. However, several groups of diatoms include terrestrial species known to occur in damp soils or ephemeral aquatic habitats, as well as more arid habitats. BSC diatoms are phylogenetically diverse but are dominated by pennate species from at least four taxonomic orders. On a broad geographical scale, diatoms occur in diverse BSC habitats on all continents (Sharma et al., 2007; Souffreau et al., 2013a; Büdel et al., 2016), and many of the same species have been found on different continents, indicating that diatoms can be transported great distances (Souffreau et al., 2013a). More locally, diatom diversity in BSCs can be high, with upwards of 50 species reported (Borchhardt et al., 2017). Unlike green algae, whose walls are carbon-based, diatom cell walls are made of silicon dioxide. Often pennate diatoms, typical of many BSC species, have a slit-like channel (raphe) along the length of the cell that produces carbon-based mucilaginous exudates that accommodate motility (Edgar and Pickett-Heaps, 1982). These mucilaginous exudates also likely contribute to soil aggregation and stabilization in BSC habitats. Motile diatoms exhibit vertical movement in BSCs, in response to light and water availability (Hu et al., 2003). A significant diversity of diatom vegetative cells isolated from damp soils have been shown to be tolerant of wide temperature fluctuations (both heating and freezing), but they were not tolerant of desiccation. On the other hand, resting stages of terrestrial diatoms were resistant to periods without water (Souffreau et al., 2010, 2013b). This ability would allow for aerial transport, and a number of viable diatoms have been detected in airborne samples (Brown et al., 1964; Sharma et al., 2007). However, the precise mechanisms used by diatoms to tolerate periods of desiccation are not well-known.

Diatom vegetative cells including other cell types relevant to dispersal and perennation (e.g., auxospores) are diploid (2n). Asexual reproduction in diatoms involves mitotic divisions of vegetative cells (Mann, 1993). Initiation of sexual reproduction is directly related to the size of vegetative cells and is typically triggered when vegetative cell size has been reduced through repeated mitotic cell divisions, to a minimum size—usually smaller than half of the normal size for most species (Edlund and Stoermer, 1997). This overall reduction in cell size, through repeated mitotic divisions, is due to the fact that as vegetative cells divide the overlapping cell halves (valves or frustules) separate with both the top (larger) and bottom (smaller) halves regenerating a new bottom (smaller) half. The specific processes associated with sexual reproduction differs between the two main groups of diatoms—centric and pennate forms. However, the outcome is similar for both groups—production of a diploid structure—the auxospore that eventually restores the vegetative

cell to the normal size and form (Kaczmarska et al., 2001). With subsequent mitotic cell divisions again reducing the size of future generations of vegetative cells to a point that sexual reproduction is triggered with meiosis producing gametes, by either oogamy or isogamy depending on the species. Gametes subsequently fuse to form the next generation of diploid auxospores (Mann, 1993).

Aquatic diatoms are generally dispersed by water currents. However, terrestrial diatoms (e.g., vegetative cells and auxospores) are typically dispersed through the atmosphere under very dry conditions (Sharma and Singh, 2010; Aguilera et al., 2018). Research has shown that diatoms can reach the atmosphere in various ways including volcanic eruptions (Pike, 2013; Van Eaton et al., 2013) and dust and sand storms (Griffin et al., 2002). Airborne diatom propagules have been collected by various methods from a variety of locations (Holzapfel, 1978; Vanormelingen et al., 2008; Pearce et al., 2016; Tesson et al., 2016).

BRYOPHYTES

BRYOPHYTES are small spore-producing plants with a dominant and normally persistent gametophyte and include mosses, liverworts, and hornworts. Although without true xylem, many species of mosses are capable of effectively conducting water both endohydrically (in specialized water conducting tissues containing xylem precursors) and ectohydrically by quickly wicking up liquid water along external cell surfaces, often in a matter of seconds from the substrate into the shoot apices (Glime, 2017). Bryophytes, similar to lichens, are poikilohydric, whereby their tissues are capable of exchanging water vapor from ambient air and equilibrating their tissue water content with the water potential of the surrounding air (Glime, 2017). Most bryophytes differ from lichens in being unable to fully activate metabolism using just water vapor, although imbibing water vapor while yet desiccated acts to mitigate desiccation damage to tissues (Pardow and Lakatos, 2013; Slate et al., 2018). Nevertheless, carbon balance can be positive and recovery complete in some mosses without the addition of liquid water (Lange, 1969; Lakatos, 2011). Between the three groups, bryophytes comprise some 20,000 species (Shaw et al., 2011). Although there is a common misconception that bryophytes occur only in damp, shady environments, some species occur in hot, dry environments, or dry polar environments (McCleary, 1959; Scott, 1982). With the exception of the liverwort genus *Cryptothallus*, virtually all bryophytes contain chlorophyll and are photosynthetic. *Cryptothallus* has no chlorophyll and gets its photosynthates indirectly from a host tree via a symbiotic relationship with a saprophytic fungus of the Basidiomycota (Wickett and Goffinet, 2008). Most bryophytes are capable of surviving desiccation during rainless periods (Proctor et al., 2007) and desiccation tolerance even extends to antheridia and male gametes (Shortlidge et al., 2012; Stark et al., 2016b), embryonic sporophytes, and asexual propagules (Brinda et al., 2016; Stark et al., 2016a). Upon addition of liquid water, shoots become metabolically active (Harten and Eickmeier, 1987) and require about 24 h to recover in most instances (Coe et al., 2014).

Bryophytes are able to anchor themselves to the ground or to other suitable substrates with slender root-like rhizoids that occur on the underside of the thallus, bases of shoots, or when protonemata become subterranean (Odu, 1978; Jang et al., 2011; Glime, 2017).

Bryophytes may reproduce sexually and asexually and these modes may co-occur in time. Although dependent upon liquid water for gamete transfer, in deserts selection has likely favored monoecious over dioecious (monoicy over dioicy) reproduction (Wyatt, 1982). A significant number of dioecious BSC mosses exist, and some of the more common species may (e.g., *Bryum argenteum*) or may not (e.g., *Syntrichia caninervis*, *S. ruralis*) produce aboveground specialized asexual propagules. Such asexual propagules may be cryptic and only produced during periods of abundant moisture, as in the protonemal gemmae of *S. caninervis*, *Funaria hygrometrica*, and *B. argenteum* (Glime, 2017). One of the most successful and widespread BSC species, *B. argenteum*, is capable of producing not only abundant spore capsules when both sexes are present, but also two kinds of gemmae (bulbils and protonemal gemmae). While the spores of *B. argenteum* are dispersed by air currents, their gemmae float and are likely instrumental in short distance dispersal by water. In addition to spores and propagules, probably all BSC bryophyte species commonly disperse by fragmentation of the plant body, the cells of which are totipotent (Glime, 2017). In their sexual life cycle bryophytes produce multicellular haploid individuals (gametophytes) which produce gametes through mitosis (Glime, 2017). Male and female gametes then fuse to form diploid individuals (sporophytes) which remain attached to the gametophytes and produce haploid spores by meiosis that in turn divide by mitosis to produce the multicellular haploid gametophyte generation—thus completing the bryophyte life cycle (Glime, 2017). Sexual reproduction in many bryophytes is uncommon in dry environments due to the paucity of sexual spores or the rarity of male plants (Bowker et al., 2000; Stark et al., 2000; Horsley et al., 2011). Asexual reproduction in bryophytes is far more prevalent than sexual reproduction (During and van Tooren, 1987; Hugonnot and Celle, 2012) and generally regarded as the primary avenue of species dispersal (Mishler, 1988). Genetic diversity of at least some BSC bryophyte species can remain high regardless of whether reproduction is sexual or asexual (Paasch et al., 2015). Asexual reproduction is more common in mosses (Pohjamo and Laaka-Lindberg, 2003) whereas sexual reproduction is more common in liverworts (Peñaloza-Bojacá et al., 2018).

Some bryophyte species are capable of producing millions of meiospores from individual sporophyte capsules or from a single m^2 (Longton, 1997; Australian National Botanic Gardens, 2016). However, the release of aerial propagules and particles can vary significantly over the period of a year based on weather conditions (Barbé et al., 2017), including wind speed and turbulence (Johansson et al., 2014). Aerial dispersal of bryophyte propagules is common (Flø and Hågvar, 2013) and long-distance dispersal rates may exceed shorter-distance dispersal (Lönnell et al., 2012). Long-distance aerial dispersal of diaspores (spores, gemmae, and/or leaf fragments) have all been reported (Laaka-Lindberg et al., 2003). Trans-oceanic

and intercontinental dispersal is common (van Zanten, 1978; Shaw et al., 2011) and contributes to the intercontinental and bipolar distribution of species (Piñeiro et al., 2012; Lewis et al., 2014; Biersma et al., 2017). Such long-distance dispersal is now regarded as explanatory for the relative rarity of narrow endemics among bryophytes and other spore-bearing organisms (Shaw and Goffinet, 2000) when compared to seed plants. Gene flow through occasional long-distance dispersal events apparently prevents the evolution of, for example, sister species on different continents. The apparent lack of connectivity of populations is due in large part to the lack of environmental uniformity. Although propagules may be widely dispersed, not all end up in a climate or on a substrate conducive to their survival. Desert soils of the world are often dominated by a relatively few, widely distributed species of bryophytes. These are characterized by strongly developed traits of desiccation tolerance, whereby a constitutive or strongly inducible ecological strategy of desiccation tolerance has evolved (e.g., the genera *Syntrichia*, *Tortula*, *Bryum, Crossidium, Aloina, Pterygoneurum*). Interestingly, an inducible strategy of tolerating desiccation characterizes some of the common arid land mosses in the latter three genera. Such strategies enable the gametophyte shoots of mosses to tolerate rapid or slow rates of drying to the very low equilibrating relative humidity typical of desert habitats. Even so, the life phases of bryophytes exhibit a range of desiccation tolerance, with protonema and juvenile structures normally less resistant to desiccation (Greenwood et al., in review). In Janice Glime's E-book chapter on diaspore dispersal in bryophytes (2017), a number of studies based on several species and lines of reasoning support the idea of long-distance transport of diaspores that in turn lends credibility to the Baas-Becking hypothesis that "everything is everywhere and the environment selects." Briefly, bryophyte species disperse farther than vascular plants, bryophyte species are generally more widely distributed than those of vascular plants, and the large numbers of disjunct bryophyte taxa between distant regions align with long-distance dispersal as the most supported process underlying these facts. The Baas-Becking hypothesis has been demonstrated for at least two bryophyte species, *Bryum argenteum and Plagiobryum zierii*, and also for the *Sphagnum* flora on an archipelago in the Baltic Sea. In addition, her review determined that spore size differences (small vs. large) does not inhibit dispersal, and that trans-continental spore dispersal is occurring. The *Sphagnum* spore rain is significant and thermal updraft and wind are significant factors controlling the dispersal of spores. Both updrafts and smoke from fires also likely facilitate spore dispersal. In the species *Discelium nudum*, evidence suggests that the majority of spores "escape the parent colony to travel greater distances" on the order of at least kilometers. Such evidence is consistent with the life history of many bryophyte species. Further, van Zanten (1976, 1978) and van Zanten and Pocs (1981) document spore survival under conditions typical of high altitude atmospheric air currents (i.e., desiccation, wetting, and freezing) and provide evidence that bryophyte spores can enter the jet stream and travel great distances, adding to other studies indicating that spores may be recovered in rainwater. Multiple genetic studies support a long distance dispersal hypothesis. Bryophyte spores are capable of surviving in the desiccated state for decades. Comparisons of spore desiccation tolerance and frost resistance between transoceanic and endemic species support long distance dispersal patterns, and the spore wall chemical composition suggests high survival capability befitting long distance transport.

SUMMARY AND DISCUSSION

Biological soil crust communities include a variety of organisms that occupy the surface layer of the soil and consolidate soil particles and aggregates into a distinguishable stable crust resistant to the erosive forces of wind and water. The most common and abundant BSC organismal groups include—cyano-, heterotrophic, and diazotrophic bacteria, free-living, lichenized, and mycorrhizal fungi, eukaryotic algae including diatoms, and bryophytes. Historically, BSCs have been most noticeable and researched in arid and semiarid environments where the vascular plant community tends to be dominated by smaller shrubs with open inter-shrub spaces that offer minimal competition for direct sunlight and provide for more equitable distribution of essential resources. BSCs are becoming more recognized in humid environments, including the humid tropics; particularly, where disturbance has temporarily removed vascular plants that normally compete for sunlight and other essential resources at soil surfaces.

Herein, we provide a thorough discussion of the various modes of reproduction and dispersal employed by the core BSC organismal groups. Modes of reproduction vary between the organismal groups but generally include both sexual and asexual alternatives, although asexual options (e.g., fission, budding, fragmentation, etc.) are far more prevalent among BSC organisms. Following successful reproduction, sexual, and/or asexual propagules must be dispersed to new, suitable environments in order to avoid competition with established local BSC communities. Almost universally, BSCs are dispersed aerially, often for very long distances, sometimes intercontinentally and interhemispherically (Muñoz et al., 2004; Sharma and Singh, 2010; Smith et al., 2013, 2018; Herbold et al., 2014; Maki et al., 2019; Warren et al., 2019).

In spite of the extensive, research concerning arid BSC communities over the last 50 years there are still areas of research that need additional work. Specifically, some of the organismal groups are poorly known—particularly in terms of species diversity and ecological roles. For example, very little is known about the diversity and ecological roles of both the free-living and mycorrhizal fungi associated with BSCs. More work is also needed in terms of better understanding the community and ecosystem dynamics of BSCs. This paper provides important background information about the occurrence, reproduction, and dispersal of the various core organismal groups that contribute to the development and maintenance of BSC communities. However, effective application of modern molecular and microenvironment sampling tools will be pivotal in answering key questions about the diversity and functional attributes and relationships within BSC communities.

AUTHOR CONTRIBUTIONS

SW and LLS initiated, organized, and directed the endeavor. They jointly composed the section on cyanobacteria. SW composed the section on mycorrhizal fungi. LLS composed the section on lichenized fungi. LRS composed the section on bryophytes. LL composed the section on terrestrial algae. JS, NP, and TK composed the section on free-living fungi. ZA composed the section chemoheterotrophic bacteria.

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Interactions of Microhabitat and Time Control Grassland Bacterial and Fungal Composition

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Dryland grasslands are vast and globally important and, as in all terrestrial ecosystems, soil microbial communities play fundamental roles in regulating dryland ecosystem function. A typical characteristic of drylands is the spatial mosaic of vascular plant cover surrounded by interspace soils, where biological soil crusts (biocrusts)—a complex community of organisms including bacteria, fungi, algae, mosses, and lichens—are common. The implications of this heterogeneity, where plants and biocrust cover co-occur, are often explored in the context of soil fertility and hydrology, but rarely has the impact of these multiple microhabitat types been simultaneously explored to determine the influence on bacterial and fungal communities, key biological players in these ecosystems. Further, our understanding of the temporal dynamics of bacterial and fungal communities in grasslands, and of how these dynamics depend on the microhabitat within the ecosystem, is notably poor. Here we used a temporally and spatially explicit approach to assess bacterial and fungal communities in a grassland on the Colorado Plateau, and to link variation in these communities to edaphic characteristics. We found that microhabitat (e.g., vascular plant rhizosphere, biocrust, and below biocrust) was the strongest driver of differences in bacterial and fungal community richness, diversity, and composition. Microhabitat type also significantly mediated the impact of temporal change in shaping community composition. Taken together, 29% of the variation in bacterial community composition could be explained by microhabitat, date, and microhabitat-by-date interactions, while only 11% of the variation in fungal community composition could be explained by the same factors, suggesting important differences in community assembly processes. Soil microbial communities dictate myriad critical ecosystem functions, thus understanding the factors that control their compostition is crucial to considering and forecasting how terrestrial ecosystems work. Overall, this case study provides insights for future studies on the spatial and temporal dynamics of bacterial and fungal communities in dryland grasslands.

Keywords: biological soil crusts, diversity, temporal dynamics, fungi, bacteria

INTRODUCTION

Semiarid, arid, and hyperarid (hereafter, dryland) ecosystems cover over 40% of the terrestrial surface across the globe (Safriel et al., 2005) and ∼35% of the western US (Pointing and Belnap, 2012). Dryland grasslands play particularly important roles in drylands: grasslands are home to many endemic plants and animals, are critical for livestock and wildlife grazing, are hotspots of recreation, and are at risk under global change (Maestre et al., 2016). In dryland ecosystems in general, and in the high deserts of the southwestern US in particular, high climate variability is the norm, with temperature and precipitation fluctuating on timescales ranging from hours to seasons to centuries (Sheppard et al., 2002). The high variability in precipitation and temperature makes it a challenging environment for species survival; despite this, a diversity of plants and microorganisms have adapted to these conditions and these organisms have helped shape the unique landscape. This landscape is heterogenous, typically comprised of sparsely distributed plants and numerous types of biological soil crusts (biocrusts) covering the interspace soils among plants (Weber et al., 2016). Biocrusts are diverse and can be formed by cyanobacteria, algae, fungi, lichens, and/or mosses (Weber et al., 2016). The heterogeneity in dryland biota (e.g., vascular plants surrounded by interspace soils; **Figure S1**) creates a variety of diverse microhabitats, each with distinct soil bacterial and fungal communities (Grondin and Johansen, 1993; Wheeler et al., 1993; Bates et al., 2010, 2012; Steven et al., 2012, 2013, 2014; Yeager et al., 2012; Patzelt et al., 2014; Mueller et al., 2015; McHugh et al., 2017). These bacterial and fungal communities are key players in determining ecosystem functioning by contributing to soil stabilization, hydrology, and nutrient cycling (Belnap, 2006; Maestre et al., 2011, 2016; Bowker et al., 2013).

Our understanding of how microhabitats structure bacterial and fungal communities in drylands is far from complete. However, compared to our knowledge of microhabitat variation, even less is known about temporal variation in bacterial and fungal composition in arid grassland ecosystems. Early studies on seasonal changes in biocrusts used microscopy to look at changes in communities in response to climatic factors (Johansen, 1984; Johansen and Rushforth, 1985). A few more recent studies have tracked temporal variation in bacterial and fungal biomass and composition in drylands using chemical techniques (Bowker et al., 2002; Bell et al., 2009); for example, seasonal differences were observed in the community composition of desert cyanobacterial soil crusts (Bowker et al., 2002; Yeager et al., 2012). However, systematic assessments of dryland grassland bacterial and fungal communities through time are exceedingly rare. Nevertheless, studies from other ecosystems, including agricultural, mesic grasslands, alpine, arctic tundra, and rainforest systems, have shown the potential for dramatic temporal changes in bacterial and/or fungal community composition (Smit et al., 2001; Lipson et al., 2002; Griffiths et al., 2003; Wallenstein et al., 2007; Bjork et al., 2008; Matulich et al., 2015; Smith et al., 2015; Kivlin and Hawkes, 2016). Studies from other ecosystems have also examined the relative impacts of microhabitat variation (most often due to differences in plant composition) compared to the effects of season on bacterial and/or fungal composition (i.e., these studies have simultaneously assessed the control of spatial and temporal variation), with varied results. In both an alpine tundra (Bjork et al., 2008) and a rainforest (Kivlin and Hawkes, 2016), soil microbial community changes over time were of greater magnitude than differences attributed to association with a specific plant community. In contrast, plant composition (tussock or shrub) was a stronger regulator of microbial communities than season in Arctic tundra soils (Wallenstein et al., 2007). The variability in the magnitude of temporal versus microhabitat effects across ecosystems is perhaps not unexpected, as temporal changes at a location may be affected by numerous environmental (e.g., temperature, soil moisture) and ecological (e.g., dispersal) factors, and these factors may be highly variable and location dependent. Nevertheless, tracking seasonal dynamics concurrently with microhabitat controls has the potential to broaden our understanding of controls over the diversity of bacteria and fungi, especially those living in extreme environments (Schadt et al., 2003).

Patchy landscapes with distinct microhabitats caused by the presence or absence of vascular plants or different soil surface covers (**Figure S1**) are ideal for examining temporal changes in dissimilar environments while keeping other factors, such as climate and land use history, constant. However, to our knowledge no study has simultaneously evaluated how dryland grassland bacterial and fungal communities vary by microhabitat type and through time, leaving interactions between these likely controls unexplored. Here, we assessed how microhabitats and time separately and together impact bacterial and fungal community composition in a semiarid grassland ecosystem. Specifically, we asked: (1) How do changes in community composition across microhabitats compare to changes across season? and (2) Do the temporal community dynamics differ with microhabitat type? We hypothesized that microhabitats would exert greater control over bacterial and fungal community composition than temporal variation. Microhabitats generally vary in pH, soil texture, sunlight/UV exposure, temperature, and other environmental factors that control the composition of the microflora (Steven et al., 2013). Furthermore, we hypothesized that within the interspace microhabitats, the below-biocrust communities would change less than the biocrust communities. This is because surface communities are likely subject both to more extreme climatic changes (e.g., Tucker et al., 2017) and higher rates of microorganism dispersal, which are buffered in subsurface soils. Understanding the relative roles of spatial and temporal variation can provide insights into the drivers of community assembly in extreme environments, and provide a baseline from which to understand the effects of anthropogenic changes on grassland bacterial and fungal communities.

MATERIALS AND METHODS

Study Site and Soil Sampling

This study was conducted in a semiarid grassland located near Castle Valley, Utah, USA and close to a long-term climate manipulation experiment on the Colroado Plateau (38°40′26.52″

N, 109°24′59.27″ W; Wertin et al., 2015). The site has a history of limited- to no direct anthropogenic impacts prior to establishment of the study and the soils are classified as sandy loam, calcareous, Rizno series (Grand County Soil Survey; Winkler et al., 2019). Air temperature and precipitation values were collected from a weather station located ∼200 m from the collection site (**Figure S1**). The average annual precipitation was 257 mm and the mean annual temperature 14.0°C during our study period (2013–2014). This was similar to longer-term averages (2006–2018) collected at the same weather station, where average annual precipitation was 236 mm and average annual temperature 14.3°C. Soil temperature was not measured at sampling locations, but in drylands, soil, and air temperatures are often highly correlated (Bell et al., 2008).

The site's vegetation was dominated by the native perennial grasses *Pleuraphis jamesii* (syn. *Hilaria jamesii;* James' galleta) and *Achnatherum hymenoides* (syns. *Stipa hymenoides* and *Oryzopsis hymenoides*; Indian ricegrass) and the exotic invasive grass *Bromus tectorum* (cheatgrass). Biocrust communities were dominated by the cyanobacterium *Microcoleus vaginatus*, the cyanolichens *Collema tenax* and *C. coccophorum*, and the moss *Syntrichia caninervis*. Soils were collected from three soil locations representing distinct general microhabitats: (1) rhizosphere samples were taken from beneath the base of the native perennial grass *P. jamesii* and the exotic invasive annual grass *B. tectorum*; (2) biocrust samples were collected from plant interspaces and included three types of biocrusts as characterized by the dominant organisms (lichen, moss, and cyanobacteria); and (3) soils underlying lichen-dominated and cyanobacteria-dominated biocrusts. At each sampling point, four replicates per microhabitat were collected. Microhabitat sampling locations within the site were randomly selected at each time point. Soil samples were taken monthly from April 2013 to April 2014, but due to extreme snow conditions, the study sites were not accessible from December 2013 to February 2014, resulting in a total of 10 sampling timepoints over the 13-month period. In addition to collecting soils for microbial community assessments, samples were concurrently collected from each microhabitat type for soil chemistry. Biocrust samples were collected at 0–0.5 cm, while rhizosphere and below-surface soil samples were collected from 0.5 to 5 cm.

To assess whether dust blowing in the area contained significant amounts of bacteria and fungi that might contribute to soil populations, we also analyzed samples collected from two nearby sites. Sample were collected using "Big Springs Number Eight" (BSNE; Fryrear, 1986) sediment collectors placed 15, 50, and 100 cm above the soil surface (Flagg et al., 2014). Samples from the three heights were pooled for microbial DNA extractions. Dust samples were collected at four timepoints during 2012 (March, July, September) and 2013 (February).

Soil Chemistry

In order to characterize soil chemical differences among microsites, bulk soil chemistry measurements were obtained for composite samples from fall (October and November 2013) and spring (April and May 2014) (e.g., organic matter, total carbon, and nitrogen; **Table S1**) as soil chemical sampling at all timepoints was beyond the capacity of the project. Spring and late summer/fall are times of year when vascular plants are most active in this region (Wertin et al., 2015), thus April–May and October–November samplings were selected in order to best elucidate differences between plant-associated vs. interspace soil characteristics. Soil chemical analyses were performed by the Colorado State University Soil, Water, and Plant Testing Laboratory (Fort Collins, CO; www.soiltestinglab.colostate.edu) using their standard "routine soil and overburden" approach. Additionally, pH was measured for all individual samples at each timepoint. This was done by suspending 1 g of sample in 10 ml of distilled water. Samples were homogenized using a vortexter, allowed to settle for ∼5 min, and pH was measured using a Mettler Toledo pH meter (Mettler-Toledo, LLC, Ohio, USA).

Molecular Methods

Total nucleic acids were extracted from 0.5 g of soil using the Fast DNA for Soil kit (MP Biomedical) according to the manufacturer's instructions (http://www.mgp.cz/files/kity/FastDNASPINkit.PDF). To quantify changes in soil microbial communities over time and across microhabitats, we examined bacterial and archaeal (hereafter referred to as bacterial) and fungal communities using Illumina high throughput sequencing. Samples were prepared for sequencing on the Illumina MiSeq platform using a two-stage protocol described previously (Mueller et al., 2015). To target the bacterial community, we amplified the V3–V4 region of the 16S ribosomal RNA gene using the F515-R806 primer pair and for the fungal communities, we amplified the LSU D2 hypervariable region using the LR22R-LR3 primer pair (Mueller et al., 2015). Paired-end 250 bp sequences were generated using the Illumina MiSeq platform. A single 12 bp barcode was generated from the combinatorial barcodes and overlapping reads were joined into a single sequence using PEAR (Zhang et al., 2014) with a minimum overlap of 20 bp. Samples were de-multiplexed using QIIME (Caporaso et al., 2010) and any sequence with a mismatch to the barcode or forward primer was removed. Additional quality filtering and OTU clustering was conducted using UPARSE (Edgar, 2013) to remove any sequence with an expected error rate >0.5 and singleton sequence putative chimeras were identified against all *de novo* sequences and eliminated using UCHIME. OTUs were delineated at 97% sequence similarity for both 16S and LSU sequences. Representative sequences for each OTU bin were classified using the Ribosomal Database Project (RDP) online classifier tool (http://rdp.cme.msu.edu/) against the RDP LSU database and the 16S rRNA gene databases (Wang et al., 2007). Sequences classified to Phylum or Domain with a bootstrap <80% were removed from downstream analysis. OTU tables with taxonomic classifications are provided for fungi and bacteria (**Tables S2, S3**). Cyanobacteria are included in bacterial OTU analysis in RDP, however, additional classification for Cyanobacteria sequences were also performed using DECIPHER (Murali et al., 2018) with the Genome Taxonomy Database (Parks et al., 2018) (**Table S4**). Bacterial and Fungal OTU tables were exported for analysis in R (www.R-project.org). Using the OTU tables, we generated a rarefied composition table, randomly drawing the lowest common number of

sequences (n = 1,023, fungi and n = 1,663, bacteria). We then calculated a Bray-Curtis distance matrix that was used in the remaining analyses (Oksanen et al., 2018). Richness and shannon diversity of rarified OTU matrix libraries was also calculated in R. Fastq files used in this analysis were deposited to the MG-RAST database (mgp87567, https://www.mg-rast.org/linkin.cgi?project=mgp87567). Data can be downloaded from MG-RAST ID links for bacteria (mgm4824928.3) and fungi (mgm4824929.3). Additionally, we have added a supplemental metadata file for deposited sequence data (**Table S5**).

Statistical Analyses

To test for differences in bacterial and fungal richness and diversity across microhabitat and sampling date and to estimate the variance explained by each factor, we used a two-way ANOVA design with microhabitat and date as main fixed factors, and a microhabitat-by-date interaction. Differences in pH across microhabitat and date were also assessed using a two-way ANOVA. For significant factors, Tukey's HSD *post-hoc* tests were used to identify which groups within these factors were significantly different from each other. Using the same factors of microhabitat, date, and microhabitat-by-date, for the multivariate metric of community composition we performed a permutational multivariate analysis of variance (PERMANOVA), using type III partial sums of squares under a reduced model with 999 permutations. We estimated the percent of variation that could be attributed to each significant term for both the ANOVA and PERMANOVA analyses (Quinn and Keough, 2002). Analyses were run separately for bacteria and fungi.

We assessed correlations between environmental parameters, including air temperature, precipitation, and pH, and community metrics (richness, diversity, and composition). For precipitation, we used the cumulative precipitation over the 2 weeks prior to the sampling date (**Figure S2**). Pearson's correlations were used for univariate community metrics (richness, diversity), while Mantel tests were used for multivariate community metrics. For Mantel tests, each environmental parameter (pH, precipitation, air temperature) was used to generate a Euclidean distance matrix, while Bray-curtis distance matrices were used for bacterial and fungal community composition.

Correlations between temporal distance and community similarity for each individual microhabitat were calculated using Mantel tests. Pairwise temporal distances were measured based on the number of days that separated each sampling time point. All statistical analyses were conducted using the statistical platform R with the packages vegan, phyloseq (McMurdie and Holmes, 2013), picante (Kembel et al., 2010), and ecodist (Goslee and Urban, 2007). $P \leq 0.05$ were defined as significant.

RESULTS AND DISCUSSION

Soil Chemistry Across Microhabitats and Time

Soil chemistry measurements showed significant differences among habitat types (**Table S1**). Organic matter (OM) ranged from 1.5 to 6.1% and was highest in moss biocrust [5.0% (fall), 6.1% (spring)] and lowest in soils below lichen biocrusts [1.6% (fall), 1.5% (spring)] (**Table S1**). Total nitrogen (N) concentrations ranged from 0.03 to 0.17% and were higher in the lichen biocrust (0.08–0.17%) and moss biocrust (0.08–0.10%) compared to the other microhabitats. Carbon (C) concentrations were highest in the lichen biocrust [2.5% (fall), 3.5% (spring)]. C:N ratios varied across microhabitat type, with higher C:N ratios in the soil beneath cyanobacteria and lichen biocrusts, and lower C:N ratios in lichen, moss and cyanobacteria biocrusts (**Figure S3**). The pH was sampled more intensively with measurements taken from each microhabitat at each sampling date. The pH varied across both microhabitats and time, but there was no interaction [two-way ANOVA; microhabitat: $F_{(6,140)} = 86.5$, $p < 0.001$, date: $F_{(9,140)} = 6.7$, $p < 0.001$, microhabitat-by-date: $F_{(54,140)} = 1.3$, $p = 1.24$] (**Figures S4, S5**).

Microhabitats Are the Strongest Driver of Differences Across Microbial Community Richness Diversity and Composition

Bacterial richness differed across microhabitat type, while fungal richness did not [two-way ANOVA; microhabitat: $F_{(7,143)} = 95.1$, $P < 0.001$ (bacteria), $F_{(7,143)} = 1.87$, $P = 0.08$ (fungi), **Figures 1A,B**]. Bacterial richness was highest in the rhizosphere soils (*B. tectorum* and *P. jamesii*) and below lichen biocrusts (**Figures 1A,B**). Despite the lack of differences in richness for fungi, both bacterial and fungal diversity (Shannon index) differed across microhabitats [two-way ANOVA; microhabitat: $F_{(7,143)} = 46.3$, $P < 0.001$ (bacteria), $F_{(7,143)} = 4.0$, $P < 0.001$ (fungi)]. Bacterial diversity was also highest for the rhizosphere soils and below lichen and was lowest for cyanobacteria biocrust and dust samples (**Figure 1C**). Bacterial diversity has previously been found as lower in biocrusts compared to surrounding soil communities (Gundlapally and Garcia-Pichel, 2006). Furthermore, cyanobacteria-dominated biocrusts generally are early in the successional sequence of biocrust organisms for these ecosystems (Belnap, 2003) and thus bacterial communities may not be as developed as in other microhabitats. Fungal diversity was lowest in the lichen crust, but similar across all other habitats (**Figure 1D**).

Supporting our first hypothesis, microhabitat type was the strongest driver of differences in community composition for both bacteria and fungi [PERMANOVA; microhabitat: $F_{(7,143)} = 13.3$, $P = 0.001$ (bacteria), $F_{(7,143)} = 5.4$, $P = 0.001$ (fungi), **Figure 2**]. Overall, microhabitat explained an average 13.3% of estimated variation in bacterial community metrics, and 7.5% of estimated variation for fungal community metrics (**Figure 3**). Such differences in microbial communities in soils separated by only a few centimeters speaks to the role of microhabitat in structuring community patterns across a range of scales (Maier et al., 2018). Further, the microbial composition, and in particular the bacterial composition of soil associated with the invasive plant species *B. tectorum*, was distinct from composition associated with the native *P. jamesii*, as well as the biocrust communities (**Figure 1**, **Figures S5–S8**). Plant invasions, such as the spread of *B. tectorum*, an exotic annual grass that now dominates a large portion of the western US, are changing

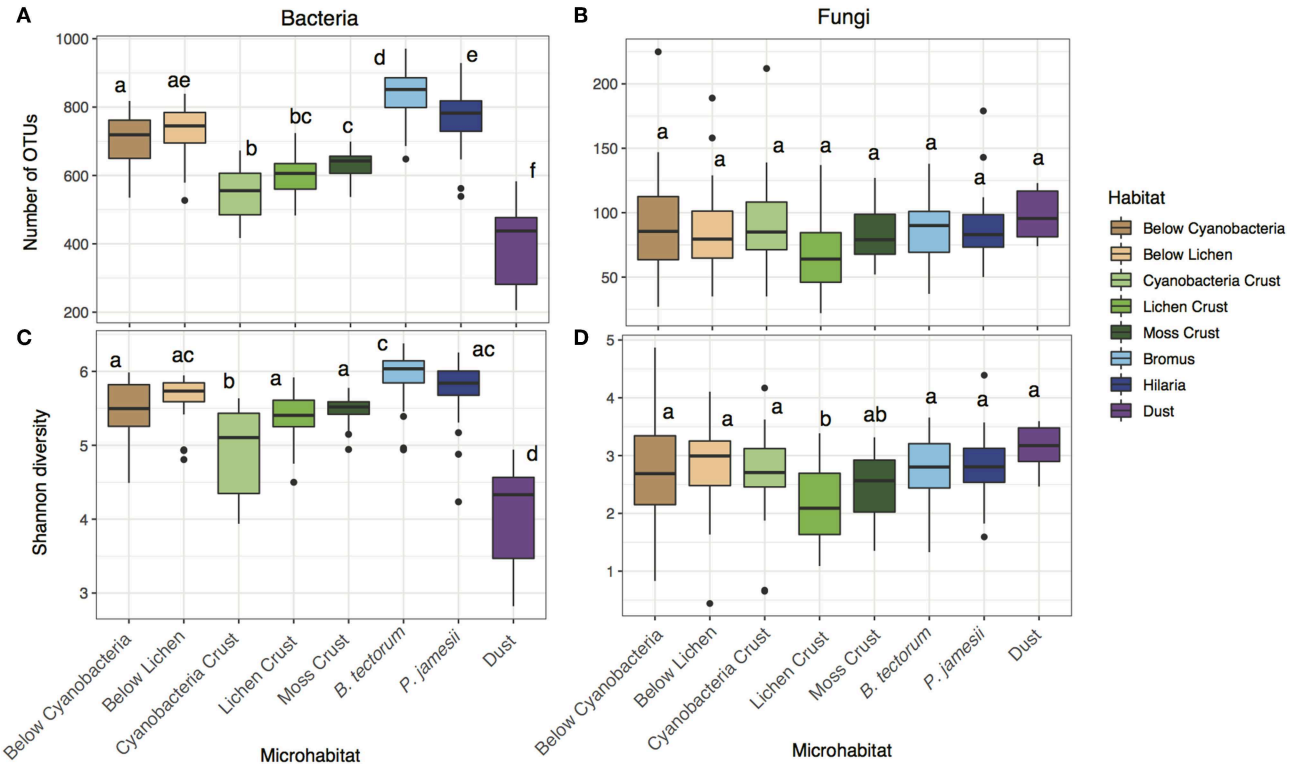

FIGURE 1 | Microhabitat variation in **(A)** bacterial richness, **(B)** fungal richness, **(C)** bacterial diversity, and **(D)** fungal diversity.

western U.S. drylands in many ways (Chapin et al., 2000; Belnap and Phillips, 2001; Compagnoni and Adler, 2014; Bradley et al., 2018). Among other influences, *B. tectorum* has altered plant biodiversity and nutrient cycling (Sperry et al., 2006; Norton et al., 2008), as well as microbial and microfaunal composition (Belnap and Phillips, 2001; Kuske et al., 2002; Belnap et al., 2005). For example, *B. tectorum* can reduce the ability of fungi to consume N (DeCrappeo et al., 2017). In addition, biocrusts have been shown to enhance *B. tectorum* growth likely through impacts on soil fertility (Ferrenberg et al., 2018). Our data support the conclusion that invasion by *B. tectorum* creates a new microhabitat type that helps dictate the extant soil microbial community.

Many fungal taxa were common across rhizosphere soils of *P. jamesii* and *B. tectorum* (**Figures S6, S8**, **Table S6**). In addition, a number of fungal genera were most highly associated with *B. tectorum,* including *Sclerotinia* (plant pathogenic fungi), *Saccobolus*, *Zygopleurage*, *Karstenula* (saprotroph), *Neofabraea* (plant pathogen/saprotroph), *Hydropisphaera*, and *Microsphaeropsis* (saprotroph) (**Figure S8**, **Table S6**), as well as the bacterial genus *Massilia*, a copiotrophic root colonizing bacteria (Ofek et al., 2012) (**Figure S9**, **Table S6**). In the biocrust communities, the fungal genus *Endocarpon* was characteristic of cyanobacteria and lichen bicrusts, but was found in much lower abundance in rhizosphere soil communities. Fungal genera *Psora, Hymenelia, and Aspicilia* were also abundant in lichen biocrusts, while *Basidiomycetes* were more common in cyanobacteria biocrusts. Moss biocrusts were characterized by *Iodophanus,* also found in the plant-associated soils, as well as *Lamprospora* and *Umbilicaria*. Generally, microbial communities in moss biocrusts were more similar to the rhizosphere soils, which is interesting given that mosses are plants and, like plant roots, have structures called rhizines embedded in the soil (Jones and Dolan, 2012). Mosses in this area fix more CO_2 compared with earlier-successional (e.g., cyanobacterial) biocrusts (Tucker et al., 2018), and thus carbon supply to the soil microbial community could be part of what supports a similar community between vascular plant roots and the soils beneath moss rhizines. Differences in the bacterial and fungal composition of biocrusts compared to non-biocrust samples may also be related to the soil depth. This study was limited to coarsely surveying complex communities through molecular techniques, future studies using additional techniques such as microscopy, culturing, and functional assays are needed to confirm the identity of and further characterize the microorganisms forming these complex communities.

Biocrusts (cyanobacteria, lichen, and moss) were dominated by Cyanobacteria and Thaumarchaeota (*Nitrososphaera*). The most abundant Cyanobacteria was *Microcoleus* (*Phormidiaceae*) (**Table S4**), which has previously been described as the most dominant cyanobacteria in Colorado plateau biocrusts (Garcia-Pichel et al., 2001). Other abundant Cyanobacterial families included *Coleofasciculaceae* and *Nostocaceae* (**Table S4**). The highest relative abundances of Thaumarchaeota were found

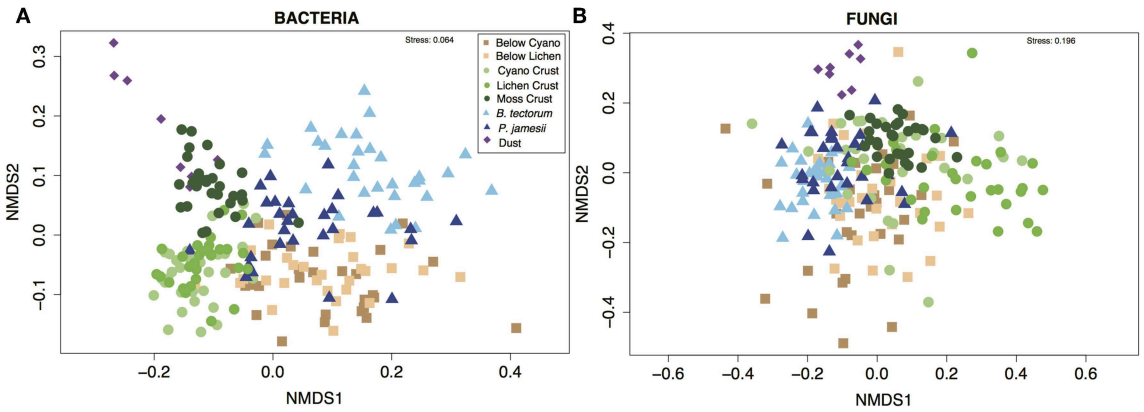

FIGURE 2 | Non-metric multidimensional scaling (NMDS) ordinations showing **(A)** bacterial and **(B)** fungal community composition across different microhabitat types.

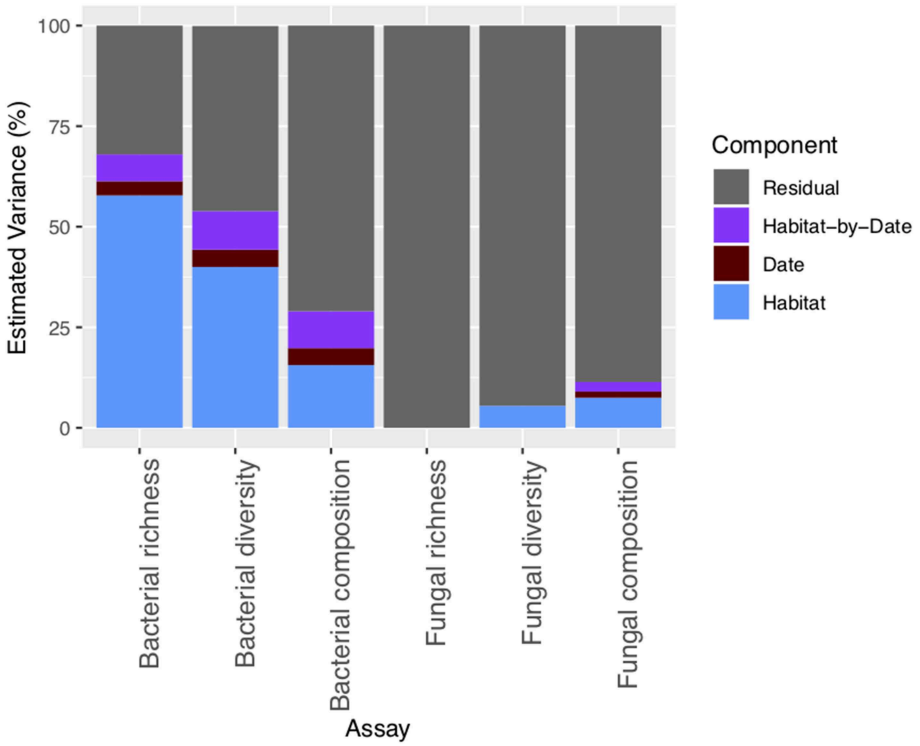

FIGURE 3 | Percentage of estimated variation across bacterial and fungal richness, diversity, and composition metrics explained by the microhabitat, date, and microhabitat-by-date.

in the soils underlying the cyanobacteria and lichen crusts (**Figure S7**), which were the most alkaline microhabitats with the highest C:N ratio (**Figures S3, S4**). Thaumarchaeota are ammonia-oxidizing archaea, that have previously been reported as significant contributors to biocrusts that may be important in soil crust N cycling (Soule et al., 2009; Marunsenko et al., 2013; Maier et al., 2018). Furthermore, previous work has found that soils ammonia-oxidizing archaea abundances are generally higher in more alkaline and NH_4^+ limited soils (Gubry-Rangin et al., 2011). A number of other abundant bacteria genera in this study including *Streptomyces, Rubrobacter*, *Paracoccus*, *Massilia*, *Bacillus*, and *Spirosoma* have also been previously characterized in biocrusts from the Southwestern U.S. Colorado plateau region (Gundlapally and Garcia-Pichel, 2006) (**Figure S9**).

Mineral soil inputs of dust are most often discussed as nutrient inputs but less often considered as inputs of biological (microbial) material. Even so, an abundance of taxa known to play important roles in biocrusts have been documented

in indoor and outdoor airborne environments (Despres et al., 2012; Barberan et al., 2015; Warren et al., 2019). We found that both the bacterial and fungal composition of dust were distinct from the soil samples (**Figure 2**). Furthermore, bacterial richness and diversity were lower in dust samples, while fungal dust samples did not differ from soils (**Figure 1**). This may be because fungal spores are generally tougher and thus less likely than bacterial cells to be degraded during the time span and harsh conditions of air travel and deposition (Kuske, 2006). Common fungal genera found in dust samples in higher abundances than in the other microhabitats we sampled included *Ochrocaldosporium*, *Thelebolus*, *Chaetosphaeronema*, and *Camarosporium* (**Figure S8**, **Table S6**). *Thelebolus* is known for ability as a thermophile to survive in extreme temperature conditions (de Hoog et al., 2005), while *Camarosporium* is known as a fungal plant pathogen (Wanasinghe et al., 2017). Dust samples were dominated by the bacterial genera *Zhihengliuella, Oxalicibacterium,* and *Hymenobacter* (**Figure S9**, **Table S7**). *Hymenobacter* has been characterized as a common atmospheric bacterium (Yooseph et al., 2013; Barberan et al., 2015). The most abundant phyla in dust samples was Deinococcus-Thermus (**Figure S7**), recognized as the most extremophilic phylum of bacteria (Theodorakopoulos et al., 2013).

Correlations Between Microbial Diversity and Environmental Parameters

Precipitation and pH were the two environmental parameters that significantly correlated with changes in microbial diversity in the grassland microhabitats, although these relationships were relatively weak. Both bacterial and fungal richness and diversity were positively correlated with pH [$R^2 = 0.34, p < 0.001$ (bacteria richness) and $R^2 = 0.13$, $p = 0.05$ (bacteria diversity), $R^2 = 0.15$, $p = 0.03$ (fungal richness), and $R^2 = 0.21$, $p = 0.003$ (fungal diversity)]. pH ranged from 6.9 to 8.4 where more alkaline soils had greater richness and diversity (**Figure 1**, **Figure S4**). Bacterial and fungal composition were significantly correlated with pH (Mantel test; $R = 0.21$, $p = 0.001$ and $R = 0.06$, $p = 0.03$, respectively). Considering climate controls, bacterial diversity was positively correlated with precipitation ($R^2 = 0.14$, $p = 0.04$), while both fungal richness and diversity were negatively correlated with precipitation ($R^2 = -0.15$, $p = 0.04$ and $R^2 = -0.14$, $p = 0.05$, respectively). These results are consistent with previous studies showing that, across global soils, changes in pH are correlated with changes in bacterial community composition (Fierer and Jackson, 2006; Rousk et al., 2010), but manipulative studies would be needed to test for causation.

Temporal Changes in Microbial Community Richness Diversity and Composition

Bacterial richness and diversity [two-way ANOVA; date: $F_{(13,143)} = 4.5$, $P < 0.001$ (richness), $F_{(13,140)} = 4.0$, $P < 0.001$ (diversity)] showed significant temporal variability, as well as a significant microhabitat-by-date interaction [two-way ANOVA; microhabitat-by-date: $F_{(54,143)} = 1.8$, $P < 0.001$ (bacterial richness), $F_{(54,143)} = 1.8$, $P < 0.001$ (bacterial diversity)]. In contrast, fungal richness and diversity showed no significant temporal variability. Both bacterial and fungal composition changed significantly across the sampling dates [PERMANOVA; date: $F_{(13,143)} = 2.9$, $P < 0.001$ (bacteria), $F_{(13,143)} = 1.5$, $P = 0.001$ (fungi)] (**Figures S6B, S7B**); however overall sampling date explained much less of the variation in community composition than microhabitat (**Figure 3**). Some fungal communities may be more stable over time than bacteria because of their extensive hyphal structures that allow them to regulate moisture availability which can change rapidly over time in dryland ecosystems (Bapiri et al., 2010; Yuste et al., 2011; Barnard et al., 2013). However, some soil fungi have also been shown to be sensitive to moisture conditions (Meisner et al., 2018).

Microhabitat Type Mediates the Impact of Temporal Change in Shaping Microbial Community Composition in a Semi-arid Grassland

For both bacteria and fungi, a significant microhabitat-by-date interaction was observed [PERMANOVA; microhabitat-by-date: $F_{(54,143)} = 1.5$, $P < 0.001$ (bacteria), $F_{(54,143)} = 1.1$, $P = 0.006$ (fungi)]. Bacterial and fungal composition were both significantly correlated with temporal distance (time between sampling points) for four out of the seven microhabitats, including cyanobacteria biocrust, below lichen, and plant-associated soil (*B. tectorum* and *P. jamesii*) (**Table 1**). In addition, bacterial composition was significantly correlated with temporal distance for the microhabitat moss biocrust (**Table 1**). These results partially support our second hypothesis that biocrust communities would change the most, and below-biocrust communities would change the least, over time. Surface soils typically experience greater climatic variability (Tucker et al., 2017), which may drive stronger temporal changes in the cyanobacteria biocrust communities. However, lichen and moss biocrust composition did not change significantly over time. This higher stability, despite the location on the surface may be because these biocrusts occur in later successional stages than cyanobacteria biocrusts (Belnap, 2003) and thus may be better established and more resistant to fluctuations in moisture and temperature. Other morphological characteristics of moss and

TABLE 1 | Correlations between community composition in each microhabitat and temporal distance (Mantel tests).

	Bacteria		Fungi	
Microhabitat	***R***	***p*-value**	***R***	***p*-value**
Cyano crust	**0.3179**	**0.001**	**0.1728**	**0.006**
Lichen crust	0.0237	0.333	−0.08984	0.943
Moss crust	**0.5311**	**0.001**	0.1083	0.069
Below cyano	0.05871	0.221	0.08592	0.128
Below lichen	**0.1789**	**0.008**	**0.1622**	**0.02**
P. jamesii	**0.2837**	**0.001**	**0.2106**	**0.006**
B. tectorum	**0.4218**	**0.001**	**0.1705**	**0.016**

Significant correlations ($p < 0.05$) are shown in bold.

lichen biocrusts may also increase resilience. The rhizosphere communities also showed a temporal correlation with bacterial community composition. The strongest correlation was with *B. tectorum*, which is an annual invasive C_3 grass that can germinate in September, expand roots over the winter, and then grow between March and April (Belnap and Phillips, 2001). The perennial C_4 grass *P. jamesii's* rhizophere microbial composition was also significantly correlated with time, but more weakly than *B. tectorum.* Temporal changes in these rhisozphere bacterial communities are likely associated with both plant life-cycles and development stage, which have been shown to influence rhizosphere communities (Houlden et al., 2008; Philippot et al., 2013; Li et al., 2014).

Differences in Semiarid Grassland Bacterial and Fungal Community Dynamics

Taken together, ∼29% of the variation in bacterial community composition could be explained by microhabitat, date, and microhabitat-by-date interaction, while only around 11% of variation in fungal community composition could be explained by the same factors (**Figure 3**). The low amount of variation explained by the environmental factors tested suggests that either fungal composition is driven by environmental factors not measured in the current study or that fungal assembly is more stochastic. Studies in other ecosystems have found that fungal communities exhibit highly stochastic assembly processes compared to bacteria, where environmental selection processes are the stronger drivers (Schmidt et al., 2014; Powell et al., 2015; Jiang et al., 2018). Our data suggest that these patterns may hold in dryland ecosystems as well.

The Future of Microbial Diversity in Semiarid Grasslands

Dryland grasslands are currently highly impacted by a variety of global changes, including altered precipitation, increased temperature and nutrient deposition, and physical disruption (Maestre et al., 2016; Plaza et al., 2018). Dryland grasslands are typically resource limited and can show low resistance and resilience to both abiotic and biotic perturbations (Barger et al., 2006; Belnap and Sherrod, 2009; Kuske et al., 2012; D'Odorico et al., 2013; Steven et al., 2018), thus the spatial heterogeneity in these landscapes is unlikely to remain static. The differences in composition across microhabitats in this study highlight the importance of spatial heterogeneity in shaping the overall diversity of microbial communities in grassland ecosystems. The microhabitats can be very close to one another, distanced by only a few centimeters or less, yet the communities observed here showed strong variation among locations and across time. While this study only examined microhabitats within a single grassland site for a single year, we expect that across larger spatial scales (multiple sites), we will see similar strong effects of microhabitat variation. The role of microhabitat heterogeneity and climatic variability in shaping diverse dryland landscapes remains an important question, and future studies that explore this variation and its consistency (or lack thereof) across multiple sites would be extremely valuable. This case study of the temporal and spatial structure of bacterial and fungal communities is one step to understanding the variability and assembly controls over microbial composition in drylands.

AUTHOR CONTRIBUTIONS

MA analyzed the data and wrote the first draft. RM analyzed the sequence data. LG-G conducted DNA extraction, PCR amplification, and prepared information for sequencing. JB, CK, and SR conceived the study and the monthly sampling.

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SUPPLEMENTARY MATERIAL

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Effects of Rate of Drying, Life History Phase, and Ecotype on the Ability of the Moss *Bryum Argenteum* to Survive Desiccation Events and the Influence on Conservation and Selection of Material for Restoration

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Desiccation stress is frequently experienced by the moss *Bryum argenteum* and can influence survival, propagation and niche selection. We attempted to disentangle the interacting factors of life history phase (five categories) and rate of desiccation (time allotted for induction of desiccation tolerance) for 13 ecotypes of *B. argenteum.* Using chlorophyll fluorescence as a stress index, we determined how these parameters influenced desiccation tolerance. Rate of drying and life phase significantly affected desiccation tolerance. The reaction norms of desiccation tolerance displayed by the 13 ecotypes showed a substantial degree of variation in phenotypic plasticity. We observed differences in survival and fluorescence between rapid and slow drying events in juveniles. These same drying applications did not produce as large of a response for adult shoots (which consistently displayed high values). Some juvenile and protonemal ecotypes, such as those from the southwest United States, possessed higher innate tolerance to rapid drying, and greater resilience compared to ecotypes sourced from mesic localities in the United States. These results show a complex nuanced response to desiccation with ecotypes displaying a range of responses to desiccation reflecting both inherently different capacities for tolerating desiccation as well as variation in capacity for phenotypic plasticity. Our results suggest that we should expect few short-term effects of climate change due to high desiccation tolerance of adult shoots, but significant adverse long-term effects on colony establishment due to low tolerance of protonema and juvenile shoots. Further, we would recommend that future studies using mosses for habitat restoration of aridlands consider the desiccation tolerance capacity of individual ecotypes used for cultivation and later re-introduction. Understanding how mosses respond to desiccation is essential to interpret ecological roles, habitat preferences, selective pressures, and responses to climate change, and to estimate the potential effects of climate changes on bryophyte species and populations.

Keywords: bryophyte, *Bryum argenteum,* chlorophyll fluorescence, desiccation, ecotype, life history, photosynthesis, rate of drying

INTRODUCTION

Desiccation tolerance throughout an organism's life cycle has emerged as an important factor not only for considering an organism's response to environmental conditions (Proctor et al., 2007; Stark, 2017) but recently for laboratory and field studies which focus on using these organisms and their unique qualities to address conservation needs or in landscape-scale rehabilitation and restoration. Bryophytes, specifically mosses, are a growing focus for use in soil and landscape restoration (e.g., Antoninka et al., 2015, 2018; Condon and David, 2016; Cruz de Carvalho et al., 2018). A desiccation tolerant organism, such as a bryophyte, can equilibrate to 50% relative humidity (RH; i.e., −100 megapascals) and resume normal function when rehydrated (Wood, 2007). Particularly in disturbed arid and semi-arid environments and considering climate change, desiccation tolerance might be key to improving materials used in rehabilitation and restoration.

There are at least two factors required for understanding and predicting survival of an organism in response to desiccation: the organism's capacity for desiccation tolerance and its capacity to improve desiccation tolerance in response to environmental cues or previous exposure. The first of these is the inherent capacity of the organism to survive a desiccating event and is unaffected by external conditions or previous drying events. The second factor, phenotypic plasticity, is the degree to which an organism increases or decreases desiccation tolerance in response to environmental cues such as previous exposure to drying events. The capacity for phenotypic plasticity undergoes ontogenetic shifts as organisms develop such that plasticity might only be observable for specific time periods. Transitions such as this have been shown in vascular plants across life cycles (Mediavilla and Escudero, 2004). Recent research has demonstrated that desiccation tolerance in mosses is a variable and plastic trait for at least some species. For example, the terrestrial moss *Physcomitrella patens* and the aquatic moss *Fontinalis antipyretica*, were generally recognized as desiccation sensitive, but studies have demonstrated these mosses can withstand desiccation if a slow rate of drying is applied (Cruz de Carvalho et al., 2011, 2014; Greenwood and Stark, 2014).

Over the life cycle of an organism, the relative importance of abiotic (e.g., desiccation, intense light, heat, etc.) and biotic (e.g., intra and interspecific competition) factors can shift. For example, juvenile shoots appear to prioritize vertical growth to maximize energy gathering ability (e.g., shade competition) similar to vascular plants allocating energy between defense, reproduction, and growth over development (Bazzaz et al., 1987). Resource allocation shifts occur in sporophytes of *Aloina ambigua*, which switch from plastically desiccation tolerant to constitutively desiccation tolerant as sporophytes develop (Stark and Brinda, 2015).

Although phenotypic plasticity is the major means by which some organisms cope with environmental pressures, a phenotypically plastic response to desiccation would not be free of cost (Valladares et al., 2007). Generation of desiccation tolerant phenotypes would require associated maintenance and production costs for protein and sucrose formation believed necessary to develop a desiccation tolerant phenotype (DeWitt et al., 1998). Within this framework, phenotypic plasticity should be favored either in an organism which inhabits an environment that experiences shifting pressures or is variable (e.g., yearly or seasonal variation in precipitation), or in an organism that is deposited (e.g., moss spore rain; Miles and Longton, 1992) in a diversity of environments (Pigliucci, 2005).

Mosses occur in all terrestrial ecosystems, from equatorial jungles to temperate forests to deserts and even Antarctica, withstanding a diverse array of extreme environmental stresses. Mosses are often an essential component of terrestrial biocrust and play many beneficial roles such as ecosystem engineers, contributing to nutrient cycling, colonizing disturbed habitats, increasing soil stability, and aiding establishment of some seed plants (Brown and Bates, 1990; Belnap, 2006; Chaudhary et al., 2009; Weber et al., 2015). Some species can survive up to 120°C for 30 minutes (Stark et al., 2009), thousands of years beneath glacial ice sheets (La Farge et al., 2013; Roads et al., 2014), and decades of continuous desiccation at room temperature (Stark et al., 2016). Understanding how mosses respond to and tolerate desiccation can help guide conservation goals (i.e., identify vulnerable populations) and selection of materials for rehabilitation and restoration practices, particularly in advance of predicted climate changes. Land managers and restoration practitioners require guidance to develop appropriate survey techniques for identifying vulnerable populations, to develop strategies to conserve these populations (Tuba et al., 2011), and to identify potential materials (Zhao et al., 2016).

In this study we investigated the relationships between ecotypic variation, life history phase, and rate of drying upon desiccation tolerance in the species *Bryum argenteum*. *Bryum argenteum* makes an excellent study system due to its cosmopolitan distribution across arid, mesic, and urban settings. This species has one of the widest distributions of any plant and is found on all continents and in diverse habitats: hot and cold deserts, temperate and polar climates, and urban settings (Flora of North America Editorial Committee, 2014). Sexually dioecious, it is capable of short or long-distance dispersal via sexually generated spores, as well as clonal dispersal over short distances via bulbils. Phenotypic plasticity has been demonstrated previously in *B. argenteum* for thermal tolerance across ecotypes and may be reasonably expected for other physiological traits (He et al., 2016). A capacity for plasticity in response to environmental signals resulting in increased desiccation tolerance would allow a wider range of genotypes to survive the altered habitats, preserving genetic variation (Matesanz et al., 2010). This persistence would permit time either for a return to climatic norms, if they are relatively short-lived, or for the development of mutations and selection of more desiccation resistant forms, if effects are longer-lived.

The methods and design presented in this paper provide a roadmap for determining the physiological response to desiccation for populations of other mosses as well as describing how populations of a cosmopolitan moss can vary in physiological response. Specifically, we examined the relationships among five different life history phases with five rates of drying to an equilibrating 50% RH for 13 ecotypes of

B. argenteum. Similar to vegetative phenotypic phases, we used bulbils (shoot tissue that differentiates into discrete propagules), protonema, and juvenile (<3 mm), intermediate (3–5 mm), and adult (>5 mm long) shoots. Rate of drying influences the length of time which is required for an organism to reach a desiccated state at low internal water content. By examining a range of drying times (i.e., time spent at sub-turgor prior to desiccation), we can determine not just if a tissue is desiccation tolerant, but the time required for inducible desiccation tolerance to manifest. We applied these factors to specimens obtained from multiple source populations (ecotypes) in order to address to what degree ecotypes deviate in their capacity to tolerate desiccation among populations and how this might influence survival in different environments.

We hypothesized that all life phases would perform best with longer drying periods and that the effect of a slow vs. rapid dry would be more prominent in juvenile tissues and protonema. We hypothesized that evolutionary history influenced ecotypic variation and that samples from arid regions (southwest USA) would display greater desiccation tolerance and a shorter time required to induce desiccation tolerance compared to populations from mesic habitats (northwest and mid-Atlantic states USA). Finally, we hypothesized that protonema and juvenile forms would be most susceptible to desiccation, requiring longer timeframes for effective inducible desiccation tolerance compared to mature forms, which would indicate these phases are more vulnerable and less resilient to changes in environmental conditions.

MATERIALS AND METHODS

Sample Collection

Specimens were gathered from herbarium collections (collected between 1999 and 2009) from a range of North American (USA) habitats showing varied seasonal and yearly temperature and precipitation. Localities include Arizona (2 locations; male and female, ♂/♀), California (3 locations; ♂/♀), Kentucky (2 locations; ♂/♀), Nevada (1 location; ♀ only), New Mexico (1 location; ♀ only), Massachusetts [2 locations; (♂/♀), and Oregon (2 locations; ♂/♀) (see **Appendix**)]. Selection of material from different locations does not necessarily indicate specimens are different genotypes. However, localities were selected with a high degree of geographic separation to reduce the likelihood of genotype replication.

Selection of Material and Cultivation

Wet-dry cycles experienced under field conditions combined with differential levels of resources and pre-existing injury and disease make assessment of desiccation tolerance or inducibly-desiccation tolerant status difficult due to hardening (Stark et al., 2014). Induction of a hardening response has been observed in gametophytes of *Crossidium crassinerve* (Stark et al., 2014), the shoots of the gametophyte of *Physcomitrella patens*, and protonema of *Funaria hygrometrica* (Werner et al., 1991). For these reasons, we used lab cultivated samples under common growth conditions to assess desiccation tolerance and to avoid variation due to unknown field hardening effects. Previous research (e.g., Stark et al., 2014), estimated approximately 1 month of cultivation was necessary to remove field effects. For this experiment samples were kept in continuous cultivation for at least 1 year with a minimum of three subcultures before experimentation was begun.

We selected three major phenological classes: (1) bulbils, propagules specialized for roles as agents of dispersal; (2) protonema, which provide lateral expansion of moss colonies through and on surface soil; and (3) shoots, the major photosynthetic structures and bulk of gametophytic tissue. Shoots were additionally divided into three developmental classes based on height (<3 mm, juvenile; 3–5 mm, intermediate; and >5 mm, adult).

Shoots were isolated and cultivated following Greenwood and Stark (2014) to remove habitat-acquired acclimation. Bulbils were collected from shoots of stock cultures growing in sand substrate. Protonema required unique cultivation as they adhere to substrate, making isolation difficult without destroying tissue. Protonema, therefore, were cultivated in a liquid media of 30% Hoagland's solution (Hoagland and Arnon, 1950). To develop protonemal cultures, test tubes (16 × 100 mm) were inoculated with a single leaf from stock cultures. Protonemata were grown at 24°C with constant light (59 μmol m^{-2} s^{-1} PAR; photosynthetically active radiation). Tubes were sealed with parafilm and inverted twice daily to increase gas exchange.

Sample Material Preparation

For each ecotype, shoots were selected when they reached target lengths (juvenile, <3 mm; intermediate, 3–5 mm; and mature, >5 mm), with five shoots used per sample unit. For protonema, material was collected from liquid stock to produce all sample units. Due to the small size of individual bulbils, 50 bulbils collected from sand substrate stock cultures were used per sample unit. All tissue material was blotted between two sheets of Whatman #1 filter paper to remove excess superficial water before placing the tissue on top of an artificial substrate (Whatman #1 filter paper) within 35-mm diameter Petri dishes.

Stress Application

The rate of drying was controlled by adding specific volumes of sterilized water to the filter paper before the addition of tissue to the dish following Greenwood and Stark (2014). Volumes were selected to attempt to encompass a variation in drying time that are representative of xeric and semi-xeric locations such that responses for individual habitats could be extrapolated if standard rainfall patterns are known for the area. Volumes for rate of drying included: 0 μL with no lid (<20 min), 0 μL lidded (<30 min), 12.5 μL lidded (1 h), 25 μL lidded (4 h), and 50 μL lidded (11 h). Controlled rates of drying were carried out in an electric benchtop relative humidity chamber (Totech Super Dry, Totech, Tokyo, Japan) set to 50% RH. The chamber was located inside of an environmental control room (R.W. Smith and Co., San Diego, CA, USA) set to 20°C and 50% RH. Samples were stored for an additional 24 h at 50% RH after equilibration to ensure all material was dried to equilibration. Verification of dry material follows procedures in Greenwood and Stark (2014). Unstressed shoots with no prior drying events

obtained from the existing stock cultures served as controls. All treatment combinations, ecotype × life phase × rate of drying, were replicated five times.

Chlorophyll Fluorescence as a Measure of Stress

Following stress application, samples were rehydrated for 24 h, transferred to leaf clips, and dark-adapted for 30 min. Status of dark-adapted (F_v/F_m) fluorescence, non-photochemical quenching (NPQ) and quantum efficiency of photosystem II (Φ PSII) were determined with a pulse modulated chlorophyll fluorometer (FMS2, Hansatech Instruments Ltd, Norfolk, UK) using the saturation pulse method (Schreiber et al., 1995). F_v/F_m is the maximum quantum efficiency of PSII under dark-adapted conditions. Non-photochemical quenching is a method to dissipate excess excitation energy as heat, and a decrease in NPQ can indicate tissue damage. Φ PSII is the quantum efficiency of PSII electron transport in the light (Genty et al., 1989), with lower levels resulting from stress or damage.

Data Analysis

All 13 ecotypes, five life phases, and six rates of drying including controls resulted in a 13 × 5 × 6 treatment design, or 390 total potential treatment combinations, $N =$ five per treatment, totaling 1,950 individual sample units. All fluorescence parameters were analyzed for significance of main treatments and all two-way and three-way treatment interactions using analysis of variance (ANOVA) in SPSS v.20 (IBM corp., Armonk, NY, USA), with life history phase, ecotype, and rate of drying set as fixed factors. For significant effects, *post-hoc* Tukey tests were applied to determine homogeneous subsets within main treatments and all significant interactions.

RESULTS

All three main treatments and all two-way and three-way interactions between treatment combinations were significant for all fluorescence ($P < 0.001$) parameters. Significance of interactions varied within and between rate of drying treatments, but results and trends were relatively consistent for fluorescence parameters. Rate of drying appeared to have had the greatest effect on fluorescence parameters, followed by life phase and ecotype (**Figures 1A–D**). For specific results per fluorescence parameter (see **Supplemental Figures 1–3** and reaction norms on (**Figures 4**–**8**).

Rate of drying contributed strongly to the variability of fluorescence parameters within phases. Among life phases (**Figure 1A**) and among ecotypes (**Figure 1D**; **Supplemental Figures 1–3**) longer rates of drying increased fluorescence. Specifically, within ecotypes, there was an increase in fluorescence between a rapid dry and a 1 or 4 h drying time, although this was variable within ecotypes (**Supplemental Figures 1–3**). After a 1 or 4 h drying time for most ecotypes, increased drying time did not continue to significantly increase fluorescence. With increased drying time there were fewer differences among life phases (**Figure 1A**; reduced slope angle). The youngest shoots required 11 h to reach experimental control values but intermediate and mature shoots for many ecotypes demonstrated a strong response over a shorter drying time. However, within the time frames examined in this study, protonemal fluorescence values did not reach protonemal control fluorescence values and only achieved low mean values after an 11 h drying time. For example, F_v/F_m only reached a mean (± SE) of 0.368 (± 0.025) after 11 h. Overall, there was a strong trend toward greater recovery after rehydration with a longer rate of drying for most ecotypes within life phases.

Generally, for all life phases, fluorescence parameters improved with slower drying rates (**Figure 1A**). Significance between life phases varied within and between ecotype, although most of the variability was limited to the protonema and juvenile shoots across rate of drying treatments (**Figures 1–5**; **Supplemental Figures 1–3**). Protonema performed the poorest in response to desiccation stress, i.e., the lowest F_v/F_m performance (mean ± standard error; 0.355 ± 0.01), compared to shoots and bulbils (**Figure 1C**; **Supplemental Figure 1**). Protonema tended to remain vulnerable to damage across the rate of drying treatments (**Figure 1A**), except for three instances with ecotypes from the Southwest US (NM, CIMA, CA1, VF; **Figure 4**; **Supplemental Figure 1**). As shoots increase in age, mean fluorescence across all treatments and ecotypes increased: juveniles (0.635 ± 0.009); intermediates (0.697 ± 0.007); adults (0.702 ± 0.006). Bulbils display the greatest fluorescence regardless of rate of drying (0.728 ± 0.003) (**Figure 1C**; **Supplemental Figure 1**) and tended to display the highest resistance to an extremely rapid dry (**Figures 1A**, **8**; **Supplemental Figure 1**). Mean values of all phases responded positively to longer drying times, showing increased values approaching or equal to control values.

Ecotype grouped into four significantly homogeneous subsets (**Figure 1D**), with most of the signal driving these groups derived from the protonemal and juvenile shoot phases (**Figures 4**, **5**; **Supplemental Figure 1**). Variation diminished over development in shoot tissues as mature shoots from all ecotypes displayed a high tolerance for desiccation (**Figure 7**). Likewise, bulbils also showed very low ecotypic variation and an extreme capacity for desiccation tolerance and did not show variation for phenotypic plasticity in response to rate of drying (**Figure 8**).

Reaction norms (ecotype by environment) for tissues showed some overall similarities as well as capacities unique to a subset of ecotypes. First, for all ecotypes examined, bulbils displayed a remarkably flat response to the rate of drying applied, exemplifying the importance of desiccation tolerance to this phase (**Figure 8**). For adult shoots many ecotypes performed well at all drying rates; however, AZ1, MA2, KY2, when dried more rapidly than 1 h performed far below most other ecotypes; if given an induction of 1 h or more they performed near the other ecotypes (**Figure 7**). With intermediate shoots most ecotypes required 1 h for development of a desiccation tolerance phenotype. Intermediate shoots from ecotypes of NM, CA2 had high values after only 30 min, while conversely AZ1 was worse than all other ecotypes with lower values at most rates of drying and requiring a 4 h period before intermediate shoots showed values close to other ecotypes given a similar drying regime (**Figure 6**). Juvenile shoots required a longer rate

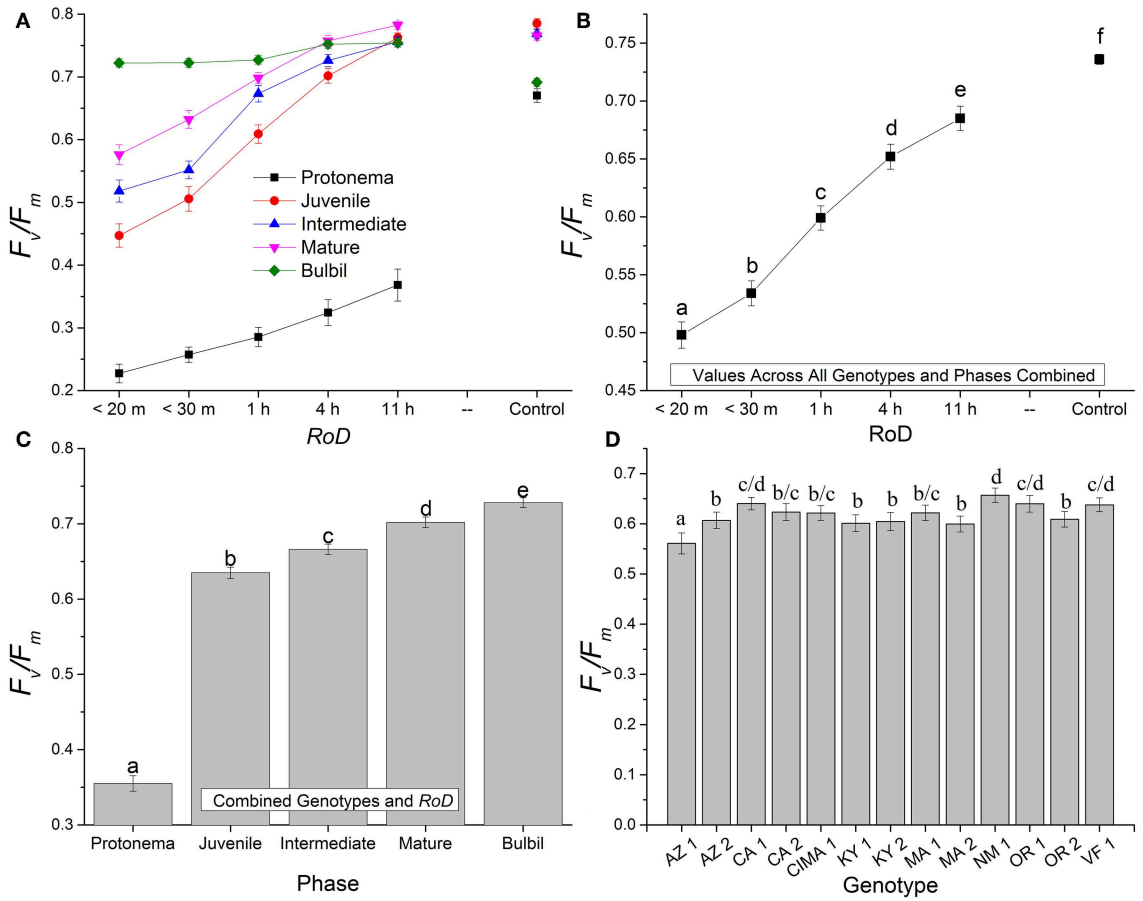

FIGURE 1 | *Bryum argenteum* F_v/F_m response variables. Panel of images showing *Bryum argenteum* F_v/F_m response variables 24 h post rehydration for the treatment combinations indicated, error bars represent ±1 SE, and lowercase letters represent homogeneous subsets established at an α of 0.05. **(A)** Two-way interaction between rate of drying (RoD) and life phase ($n = 65$), **(B)** Main effect of RoD among life phase and ecotypes combined ($n = 390$), **(C)** Main effect of life history phase among RoD and ecotype ($n = 390$), **(D)** The effect of ecotype with all life phases and RoD treatments combined ($n = 150$). RoD, Rate of Drying.

of drying to induce desiccation tolerance than other shoots on average with most ecotypes requiring one or more hours. Juvenile shoots of the AZ1 ecotype were again much less tolerant of desiccation than other ecotypes, requiring 4 h to induce a desiccation tolerance phenotype and displaying values consistently lower than all other ecotypes (**Figure 5**). Protonemal forms were the least likely to show a desiccation tolerance response of any kind in response to rate of drying, with only a handful showing signs of induction. Protonemal ecotypes of CIMA, VF, NM and CA1 were the exceptions with all showing induction after 11 h of drying, with NM and VF having reached control values. Protonema from NM showed a more rapid response than other ecotypes with strong induction achieved with only a 1 h slow dry, and control values reached by 4 h (**Figure 4**).

DISCUSSION

One might imagine two scenarios with respect to changing climate on moss distribution and species survival and how these factors relate to conservation efforts and selection of materials for restoration. In the first, a species is phenotypically plastic for desiccation tolerance, resulting in a relatively minor effect on moss residence or occupancy as a result of changes in climate conditions. A second scenario would be ecotypes that are highly adapted to their local environment possessing low phenotypic plasticity, such as we observed in protonema and juvenile shoots displaying ecotype-specific responses. We might hypothesize that a population will experience a loss in cover with a change in precipitation amount or frequency. Given these possibilities, we suggest land managers combating bryophyte cover loss to consider a pilot experiment to test the resilience of their local populations and select material that is more desiccation tolerant and resilient for restoration efforts.

Populations of *B. argentum* displayed different inherent capabilities for desiccation tolerance which are modulated by environmental factors, time spent at sub-turgor, ecotypic variation, life history phase, tissue type, and we expect a host of other environmental factors (e.g., temperature, rain frequency, solar irradiation, etc.), interacting to determine desiccation

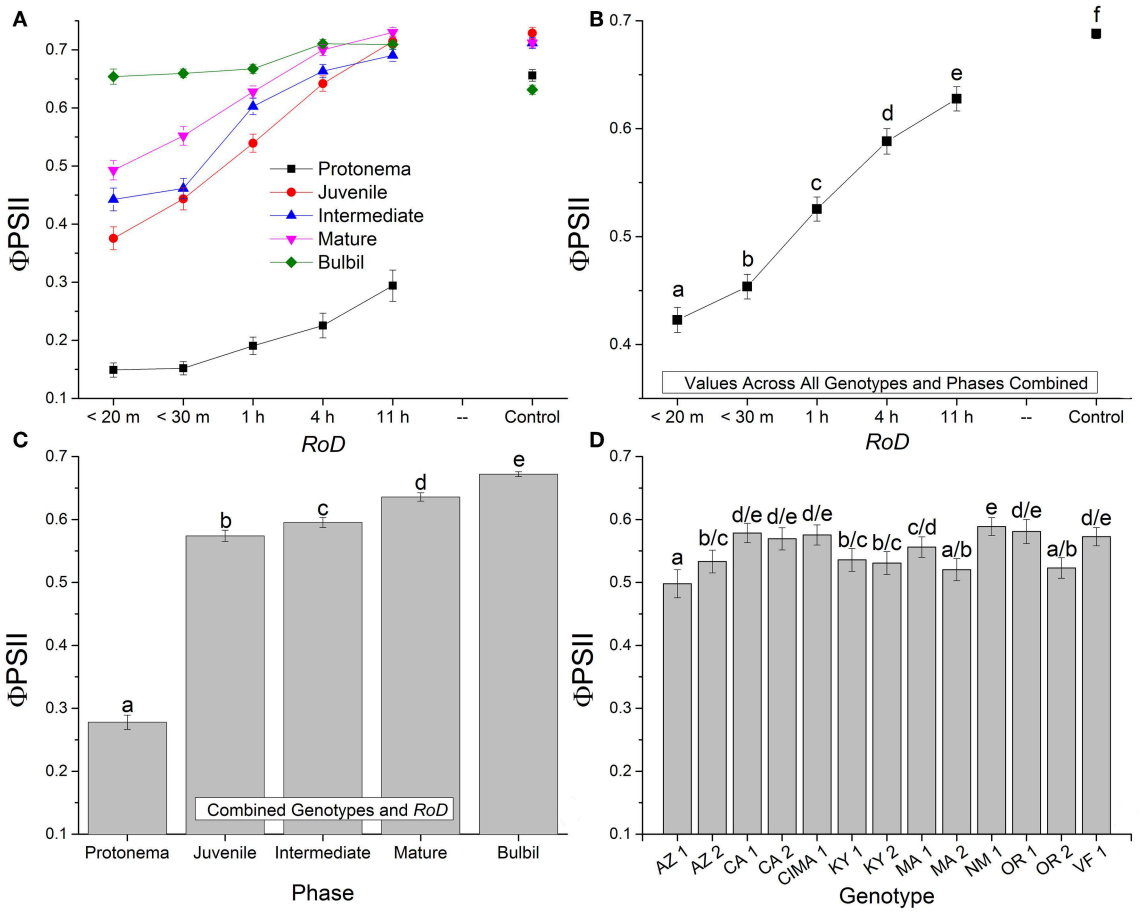

FIGURE 2 | *Bryum argenteum* Φ PSII Response variables. Panel of images showing *Bryum argenteum* Φ PSII response variables 24 h post rehydration for the treatment combinations indicated, error bars represent ±1 SE, and lowercase letters represent homogeneous subsets established at an α of 0.05. **(A)** Two-way interaction between rate of drying (RoD) and life phase ($n = 65$), **(B)** Main effect of RoD among life phase and ecotypes combined ($n = 390$), **(C)** Main effect of life history phase among RoD and ecotype ($n = 390$), **(D)** The effect of ecotype with all life phases and RoD treatments combined ($n = 150$). RoD, Rate of Drying.

tolerance. We uncovered significant differences in responses in every stage examined, showing a considerable and unexpected ontogenetic plasticity for desiccation tolerance: protonema were very resistant to induction, while bulbils exhibited extreme tolerance to desiccation at all drying rates examined. A highly variable capacity in phenotypic plasticity was found among ecotypes examined, especially within the protonemal and juvenile shoot phases. Although evidence in the literature suggests some moss species have morphological and physiological plasticity (e.g., Rincon and Grime, 1989; Hassel et al., 2005; Gao et al., 2015), few studies go beyond discussing desiccation tolerance within the context of plasticity (e.g., Stark, 2017) or measuring artificially-applied stressor responses across life history phases to test for potential phenotypic plastic responses to desiccation (but see Buryová and Shaw, 2005).

This study increases our understanding of factors related to desiccation (i.e., inducible desiccation tolerance, hardening, ecotypic, and life phase variation in desiccation tolerance) and enhances our ability to make reasonable predictions for how organisms will respond to climate changes. Predicting survival in a changing climate depends upon knowing if species have sufficient genetic variation for selection to act upon (Ghalambor et al., 2007; Matesanz et al., 2010; Shaw and Etterson, 2012). Species with high genetic variability possess a greater range of traits for selection to act upon, increasing the likelihood that some individuals in a population will be sufficiently adaptive to new climate regimes. However, if current genetic variation is insufficient for a population to adequately respond to rapid climate change, high levels of phenotypic plasticity alternatively act as a buffer against selection and allow for a wider range of existing genotypes to persist until sexual recombination can provide more competitive combinations of alleles and chromosomes (Ghalambor et al., 2007; Matesanz et al., 2010; Shaw and Etterson, 2012).

Although we observed variation in desiccation tolerance and phenotypic plasticity in desiccation response, at least for some ecotypes, this could be counter balanced by low rates of sexual reproduction and low occurrence of male sex expression in xeric

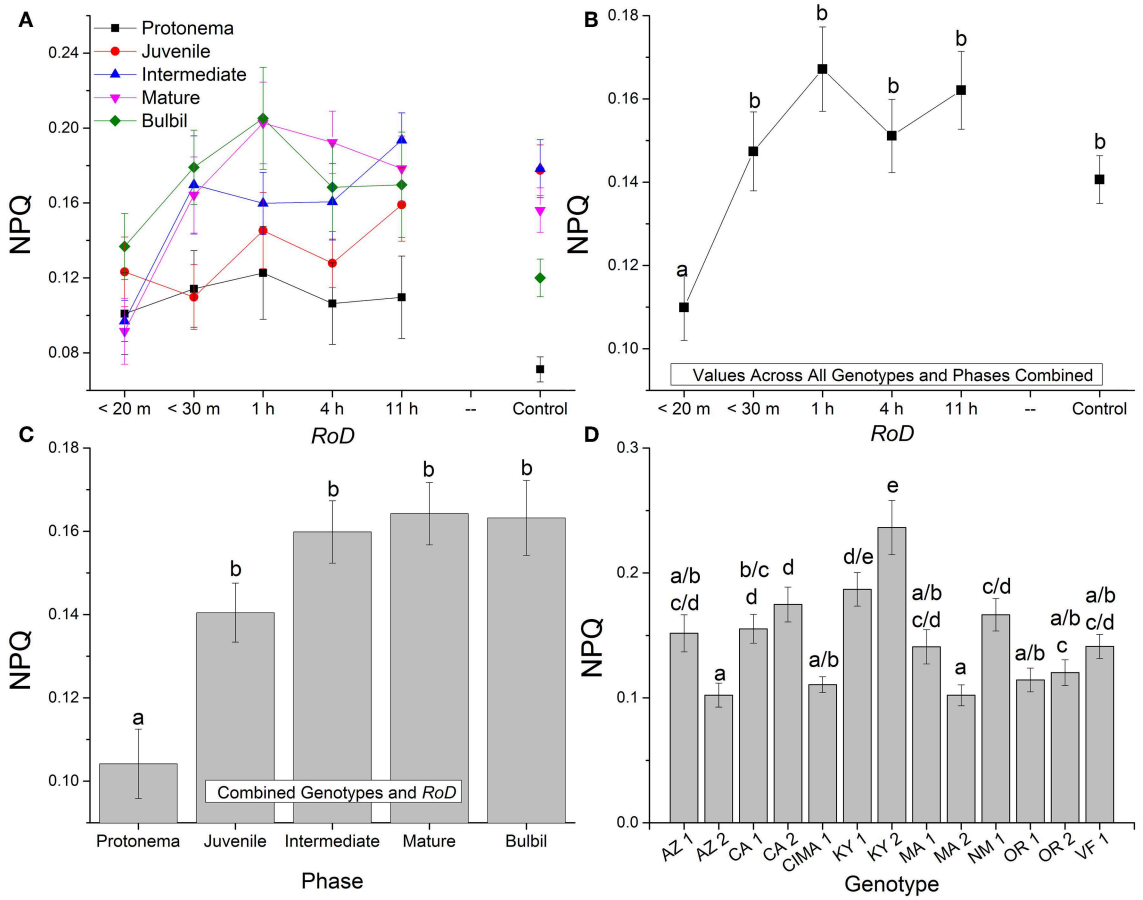

FIGURE 3 | *Bryum argenteum* NPQ response variables. Panel of images showing *Bryum argenteum* NPQ response variables 24 h post rehydration for the treatment combinations indicated, error bars represent ±1 SE, and lowercase letters represent homogeneous subsets established at an α of 0.05. **(A)** Two-way interaction between rate of drying (RoD) and life phase ($n = 65$), **(B)** Main effect of RoD among life phase and ecotypes combined ($n = 390$), **(C)** Main effect of life history phase among RoD and ecotype ($n = 390$), **(D)** The effect of ecotype with all life phases and RoD treatments combined ($n = 150$). RoD, Rate of Drying.

habitats (Stark et al., 2010). The effects of clonal propagation combined with strong selective pressure for desiccation tolerant phenotypes will likely lead to a loss of genetic variability over the short term in xeric habitats as hardier clonal forms expand into locations previously occupied by less desiccation tolerant forms. The cosmopolitan distribution of *B. argentum*, as well as its high capacity for long distance spore dispersal, over longer time frames should re-introduce genetic variation to xeric habitats. However, many other xeric species with restricted and or patchy distribution combined with potentially lower capacity for spore dispersal would have a less optimistic long-term outlook as they would not have a large source of genetic material preserved in other habitats to re-introduce into the xeric localities. We do, however, hypothesize that some species with a high capacity for dispersal are poised to act akin to an invasive species in the event of climate change, such as those like *B. argenteum* in population size, dispersal capacity, and a wide habitat preference.

Bryophytes have two major methods for dispersal: vegetative propagules (e.g., bulbils) and spores. Vegetative propagules have greater mass and density and contribute to short distance dispersal and maintenance of populations. Spores contribute to long distance dispersal across landscapes (Patiño and Vanderpoorten, 2018). Bryophytes are thought to be efficient spore-dispersing organisms, with 100 kilometers suggested as a likely distance a spore could travel (Vanderpoorten et al., 2019). Bryophytes have relatively low rates of endemism compared to seed plants, and species composition patterns match more closely with wind connectivity patterns than geographic proximity (Patiño and Vanderpoorten, 2018). With this in mind, major factors controlling dispersal ability appear to be a combination of spore mass, density, surface area, and ornamentation (Zanatta et al., 2016). A world-wide spore rain combined with the ability to succeed in many habitat types and rapid environmental shifts could result in a rapid spread of newly invasive organisms.

Desiccation Tolerance Along a Continuum of Life History Phases

The tissues examined in this study showed a wider degree of responses than expected, but all tissues responded in a manner logically consistent with their roles. Protonema are

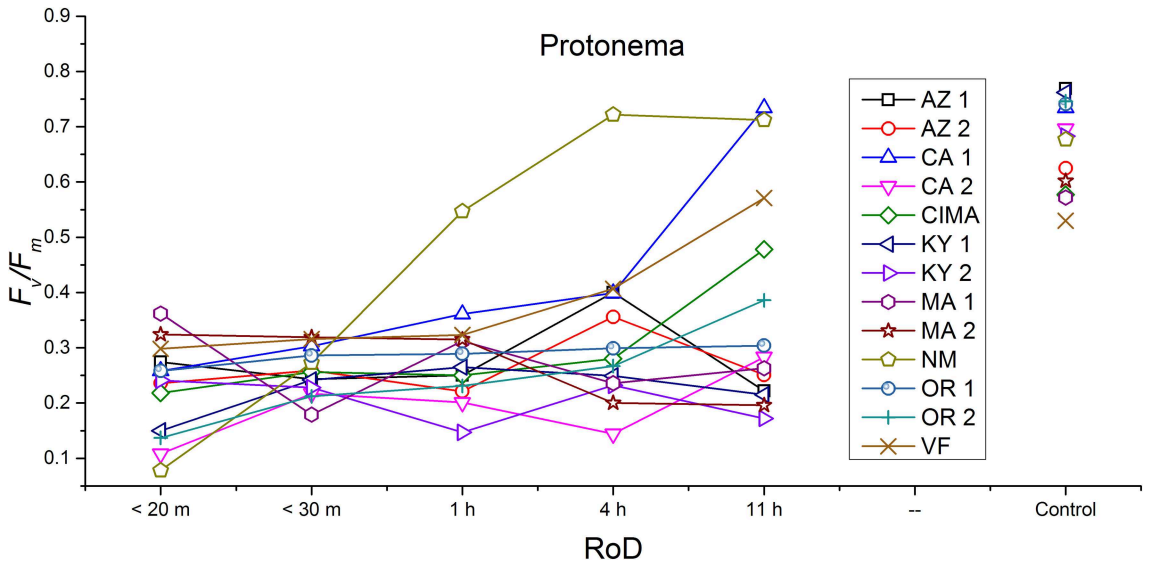

FIGURE 4 | Reaction norms for protonema from 13 ecotypes exposed to five rates of drying (RoD) 24 h post rehydration.

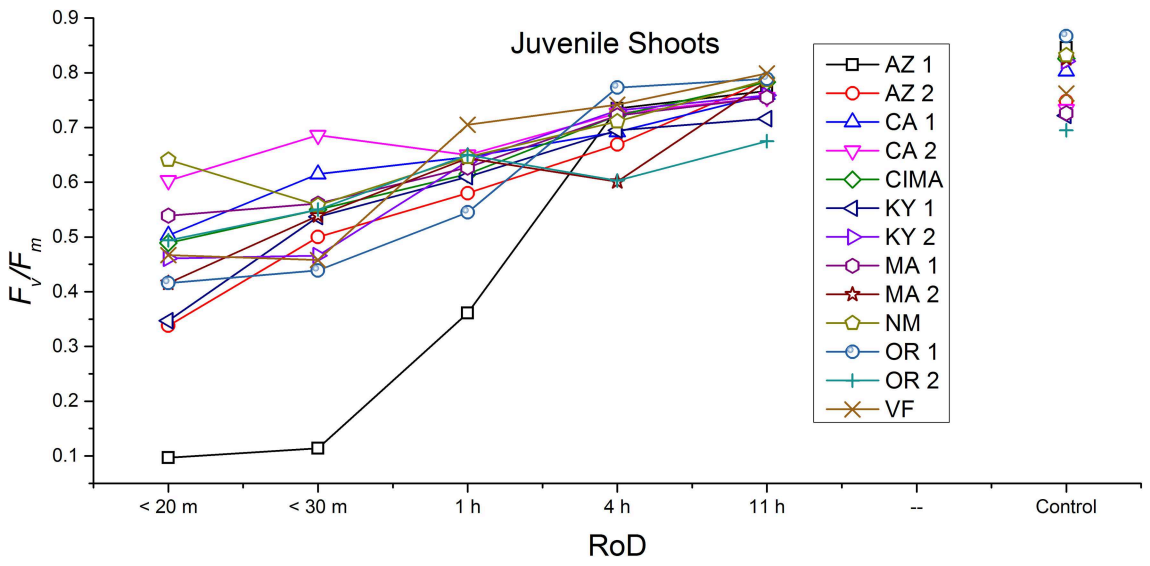

FIGURE 5 | Reaction norms for juvenile shoots from 13 ecotypes exposed to five rates of drying (RoD) 24 h post rehydration.

most sensitive. This sensitivity may be mitigated in nature as the intrinsic properties of soil result in slower drying rates than tissues aboveground, providing longer drying periods and, thus, more time for plasticity induction. Bulbils lack protective functions of colonial patch dynamics (e.g., slowing of water loss due to dense cushions) once separated (Zotz et al., 2000). However, it is not unexpected that bulbils would display an inherently high desiccation tolerance. Any dispersal agent functioning in a xeric habitat should be under strong selection for desiccation tolerance.

Moss spore production ranges from 100 million to tens of billions per square meter of moss cover annually, and any spore potentially traversing hundreds to thousands of kilometers before settling (Miles and Longton, 1992). Moss persistence would indicate a highly plastic capacity for desiccation tolerance or an inherent desiccation tolerance across vulnerable life history phases, assuming costs for maintaining a rapid plastic response are low and/or only expressed in the appropriate environments (Pigliucci, 2005). As spores have high dispersal capacity yet lack a method to control the location of deposition,

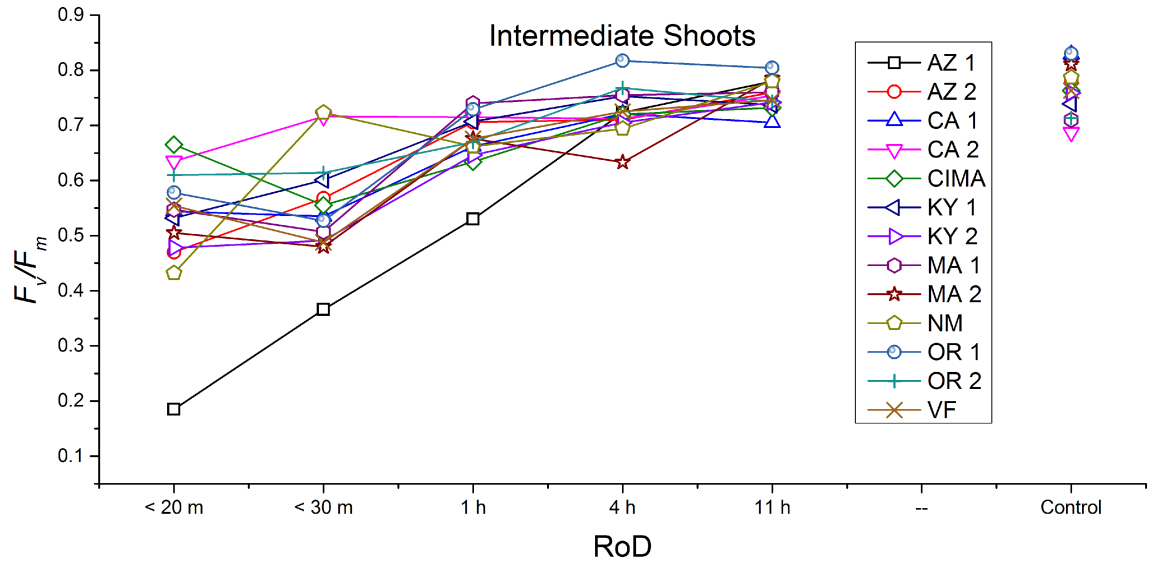

FIGURE 6 | Reaction norms for intermediate shoots from 13 ecotypes exposed to five rates of drying (RoD) 24 h post rehydration.

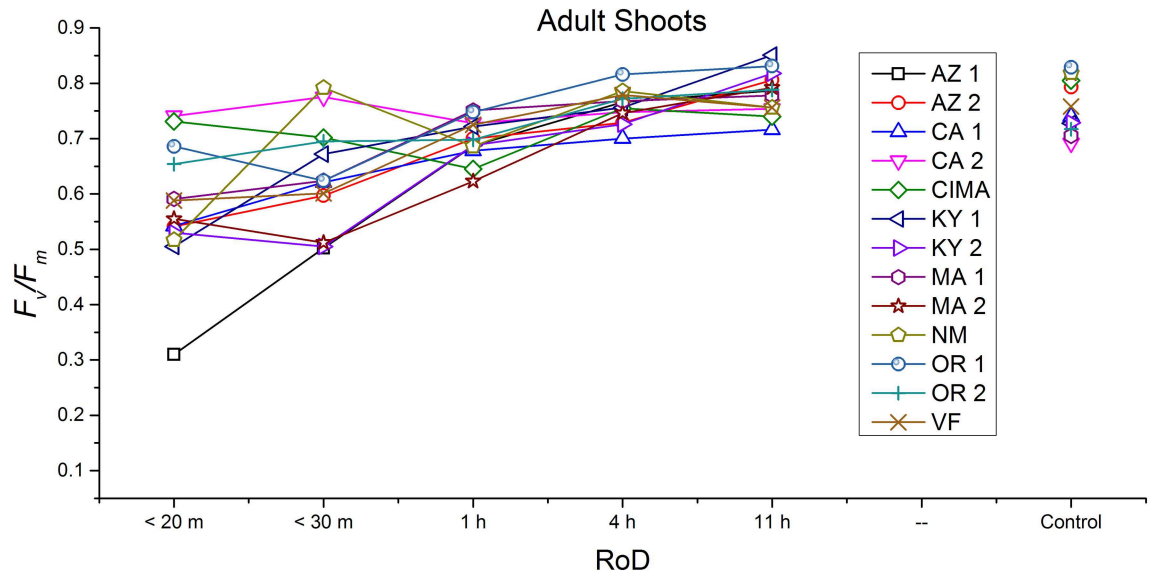

FIGURE 7 | Reaction norms for adult shoots from 13 ecotypes exposed to five rates of drying (RoD) 24 h post rehydration.

research has long suggested mosses act in accordance with the Baas Becking hypothesis *"Everything is everywhere, but the environment selects"* (Baas Becking, 1934). This agrees with studies of the genetic structure of moss populations, which have shown high genetic diversity (Skotnicki et al., 1998). An alternative explanation is that high levels of phenotypic plasticity have been selected for in high dispersal species, rendering environmental selection and ecotypic variation to a specific habitat a moot point (Pigliucci, 2005). Aspects supporting high phenotypic plasticity include the wide range of habitats inhabited by *B. argenteum* and its cosmopolitan distribution (Shaw et al., 1989).

Our results suggest that adults and established colonies are more resistant to the effects of altered rainfall patterns that are hypothesized in climate change models due to the high capacity for desiccation tolerance in adult tissue when given short drying times. However, over longer time frames (i.e., years to decades) in xeric habitats or under increasingly xeric conditions, establishment rates of new colonies or expansion of existing colonies could decrease.

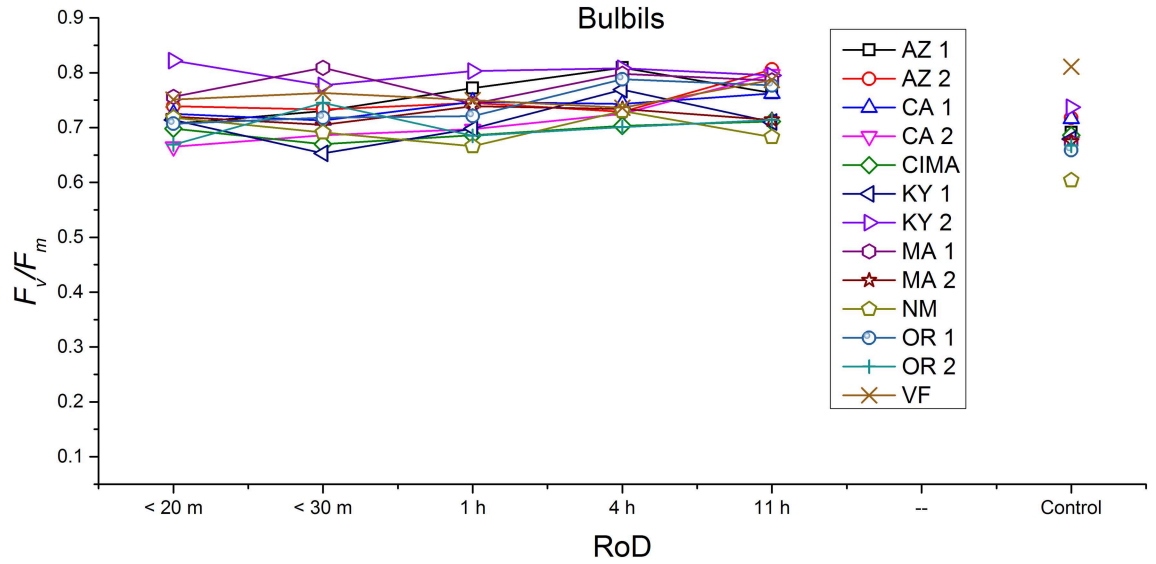

FIGURE 8 | Reaction norms for bulbils from 13 ecotypes exposed to five rates of drying (RoD) 24 h post rehydration.

Ladrón de Guevara et al. (2018) observed over an 8-year study that moss presence and abundance increased with warming through time, although growth rate reduced over time and the overall impact on the biocrust community was negative.

Our results also suggest that patch establishment and expanding regions of growth of a patch are sensitive to environmental conditions. Establishment or new growth of *B. argenteum* under xeric conditions appears strongly limited by the ability of protonema and juvenile shoots to survive drying. Because protonemal and juvenile tissue show greater sensitivity to rapid desiccation, patch establishment and expansion would likely only occur during wet seasons or favorable years and patches would likely decline under unfavorable conditions. If the rate of successful colony establishment falls beneath the rate at which mature colonies are lost, the cumulative effects would result in either expatriation from localities transitioning to more xeric habitats or a strong selective pressure resulting in increased tolerance across many populations. In general, for mosses, slow drying results in a altered protein profiles during both dehydration and rehydration and greater survival after a desiccation-rehydration cycle (Bewley, 1995; Cruz de Carvalho et al., 2011, 2014), while rapid drying leads to chlorosis and damaged photosynthetic machinery, increased reactive oxygen species production, and protein leakage all of which could be causative mechanisms behind the damage observed (Schonbeck and Bewley, 1981; Cruz de Carvalho et al., 2012, 2014; Stark et al., 2013). The diversity of inherent desiccation tolerance exhibited by *B. argenteum*, as well as plasticity of response to rates of drying, suggest *B. argenteum* might respond to selective pressures resulting in greater or accelerated selection of tolerant phenotypes (Carlson et al., 2014).

During development, mosses might transition from a plastic form of desiccation tolerance to an inherent protection, reducing the time required for inducible desiccation tolerance (Stark and Brinda, 2015; Stark et al., 2016). *Bryum argenteum* has previously displayed an ability to quickly respond to a changing environment (e.g., Raudenbush et al., 2018). Evolution selects for phenotypic traits in a population that improve the odds of survival and reproduction across life phases while minimizing expenditures. Desiccation tolerance likely has associated energy costs; therefore, it is expected that desiccation tolerance is regulated over development to reduce energy expenditure while maximizing fitness (Stearns, 1989). Factors that guide the expression of traits are the efficient utilization of limited energy resources, habitat or ecosystem conditions, genetic inheritance from ancestors, the ability to deal with stress at various life history phases, or a combination of these factors. We would expect that organisms will invest greater energy toward desiccation tolerance in habitats subjected to desiccation. However, if rates of gene flow with organisms from mesic habitats are high this might not hold true.

The reaction norms displayed by the 13 ecotypes showed a substantial degree of variation in phenotypic plasticity for protonemal and juvenile phases. The variation at these stages likely reflects increased competition during colony initiation and along the expanding edge of established colonies. In mesic habitats, energy expenditure toward a desiccation tolerant phenotype would provide little advantage, but the more rapid establishment of a colony (and the slowed water loss associated with cushion dynamics) and more rapid transition from a juvenile to an adult phase due to rapid growth would provide advantages. In habitats more likely to experience rapid and extreme drying events, any advantage gained from rapid growth at the cost of desiccation tolerance would be easily offset by the

occurrence of a single unexpected rapid drying event, favoring rapidly inducible phenotypic plasticity as seen in some ecotypes.

Conservation and Selection of Materials for Restoration

The results of this work provide an experimental framework for both conservation and restoration activities of degraded ecosystems. An ecotype specifically adapted to an environment and more sensitive to changes in a precipitation regime could perish if the home locality experienced long-term change in rainfall patterns that alter tissue drying rates below survivable levels. As a result, the important roles which mosses play in environments are likely threatened, particularly in ecosystems in which mosses contribute to significant ecological functions (e.g., Barros and Field, 2014). Understanding the degree of desiccation tolerance (plastic and inherent) and predicting how mosses will respond to climate change by assessing vulnerability help to predict which ecosystems or populations are most vulnerable. Few studies have evaluated the capacity of moss biocrusts to respond to changes in climate (e.g., Delgado-Baquerizo et al., 2016). One notable example is Robinson et al. (2018) which found a shift in the community composition of Eastern Antarctic mosses toward species which are more desiccation tolerant over a 10-year period from 2008 to 2018. Our results suggest that some moss populations are likely more vulnerable to altered rainfall patterns than others. Previous work has also suggested the vulnerability of existing moss populations (Stark et al., 2011; Reed et al., 2012). This experimental framework could assist land managers to identify vulnerable populations and identify populations within landscapes that can be used for mitigation or restoration actions, such as selection of ecotypes for cultivation and transplantation.

Because non-climate related anthropogenic effects (e.g., habitat destruction and fragmentation) are already damaging many moss populations, resources and guidance are necessary to offset degradation through restoration. Some moss species are cultivated easily in a laboratory (e.g., Greenwood and Stark, 2014; Cruz de Carvalho et al., 2018). There are several studies examining how to improve cultivation (e.g., Xu et al., 2008; Antoninka et al., 2015; Cruz de Carvalho et al., 2018). Antoninka et al. (2015) observed a 6-fold increase in 4 months of biocrust material containing mosses providing a valuable tool for outplanting. Field studies are limited but studies from desert ecosystems in China suggest mosses can be used as a component for soil stabilization and restoration (e.g., Bu et al., 2013). Utilizing mosses in biocrust restoration in cool deserts is being investigated (Antoninka et al., 2015, 2018), but few studies have used mosses in restoration applications particularly in warm deserts (e.g., Chiquoine et al., 2016; Mojave Desert, North America). Most biocrust restoration work has focused thus far on an algal or cyanobacteria components (e.g., Bu et al., 2013). To the authors' knowledge as of this writing, no published study in warm arid or semiarid ecosystems has incorporated laboratory-cultivated mosses in field studies or restoration efforts.

Selecting ecotypes for cultivation and transplantation that show a high capacity for desiccation tolerance in protonema and juvenile shoots when raised in culture could increase outplanting success of moss material. To increase successful incorporation of mosses into field applications, we suggest the following three strategies to improve outplanting attempts. First, we recommend cultivation to continue until shoots and colonies are fully mature before outplanting; second, harden samples with a slow drying event before outplanting to increase the plastic capacity for desiccation tolerance; and third, limit outplanting to cooler, wetter seasons, as this allows an entire season for new growth and adaptation to the local climate before desiccation stresses are encountered.

Further, we recommend that future restoration studies in aridlands consider the desiccation tolerance capacity of moss ecotypes used for cultivation and later re-introduction. Selection of a rapidly cultivated ecotype is tempting. However, the ability to survive transplanting and reduce field mortality rates should be a higher priority. A simple pilot study before the main effort of cultivation is begun should be employed to select for a balance of growth rate and desiccation tolerance leading to more successful re-introduction rates over the long term.

CONCLUSION

This study presents a pattern of ecotypic reaction norms to desiccation along a developmental trajectory. The use of two tissues with specialized adaptive roles (i.e., bulbils, protonema), as well as shoot tissue across multiple developmental phases, presents how an entire vegetative life cycle responds to desiccation. We examined not just whether the organism is desiccation tolerant, but also how different life history phases and tissue types prioritize rapid-to-slow induction times to achieve desiccation tolerance. We observed a gradient of responses, thereby allowing predictions of how organisms may respond to future desiccation challenges across life history phases under a variety of scenarios. An extrapolation of our results suggests that we should expect few short-term effects due to high desiccation tolerance of adult shoots, but significant adverse long-term effects on colony establishment due to low tolerance of protonema and juvenile shoots. If these trends hold true for mosses in general, species with reduced dispersal capacity in stressed ecosystems could prove disastrous. In the future, we hope results from this study will contribute to other disciplines, including ecological restoration and land management.

The overall plasticity shown for *B. argenteum* is encouraging and displays greater variation than expected, especially given only 13 ecotypes were examined. The plastic variation in response, current wide species distribution, both geographic as well as habitat variety, ease of long-distance dispersal (via spores) and short distance dispersal (spores and bulbils) suggest that the species may be better poised to respond to climate change than most (Kopp and Matuszewski, 2014). The observed variation in response among tissue types may indicate that the associated benefits of plasticity will also give *B. argenteum* a strong advantage against other species in colonizing new habitats formed by climate change, potentially allowing it to outcompete more narrowly restricted species of mosses both in terms of geographic range and suitable habitats. A quickly dispersing species such as *B. argenteum* could rapidly fill new niches as they appear, acting as a highly invasive organism and

excluding more slowly dispersing species by occupying these niches, further accelerating the loss of more vulnerable species as they are outcompeted.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

JG developed and conducted this study, analyzed the data, and primarily wrote the manuscript. LS aided in experimental design and editing of manuscript. LC aided in writing and editing of 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/fevo.2019.00388/full#supplementary-material

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Comparison of Microphototrophic Communities Living in Different Soil Environments in the High Arctic

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The Arctic region undergoes rapid climate change resulting in soil warming with consequent changes in microbial community structure. Therefore, it is important to gain more knowledge on the pioneer photosynthetic microorganisms and their relations to environmental factors. Here we provide a description of the community composition of microbial phototrophs in three different types of soils in the High Arctic (Svalbard): vegetated soil at a raised marine terrace, biological soil crust (BSC) at high elevation, and poorly-developed BSC in a glacier foreland. The studied sites differed from each other in microclimatic conditions (soil temperature and soil water content), soil chemistry and altitude. Combining morphological (cell biovolume) and molecular methods (NGS amplicon sequencing of cyanobacterial 16S rRNA and eukaryotic 18S rRNA sequences of isolates), we studied the diversity and biovolume of cyanobacteria and eukaryotic microalgae. The results showed that cyanobacteria prevailed in the high altitude BSC as well as in pioneering BSC samples in glacier foreland though with lower biomass. More specifically, filamentous cyanobacteria, mainly *Leptolyngbya* spp., dominated the BSCs from these two localities. In contrast, coccoid microalgae (green and yellow-green algae) had higher biovolume in low altitude vegetated soils. Thus, the results of this study contribute to a better understanding of microphototrophic communities in different types of Arctic soil environments.

Keywords: microbial phototrophs, the Arctic, biological soil crust, vegetated soil, diversity, microclimate, soil chemistry

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INTRODUCTION

The Arctic is characterized by the presence of continuous permafrost with exceptions in some areas (e.g., Kola Peninsula) (ACIA, 2005). The development of Arctic soils is dominated by cryogenic processes, which are driven by the formation of ice in the soils. Arctic soils have a wide range of textures, including clay, silty clay, loam, sandy loam and coarse gravelly sand, mainly depending on the mode of deposition of the parent material. Ice formation physically changes the environment and can cause biologically significant chemical changes (in salinity, pH, conductivity, and gas content). Moreover, the presence and activity of Arctic soil organisms are greatly limited by the scarcity of liquid water (Yoshitake et al., 2010). Liquid water is produced by snowmelt or thawing of the active layer in spring and summer, resulting in increased biomass of the soil communities in this period (Belnap and Lange, 2003).

The High Arctic is mostly occupied by polar deserts and/or semi-deserts and has a low vegetation cover (<25%) which includes vascular plants such as *Salix polaris*, *Papaver radicatum*, *Draba subcapitata*, *Saxifraga cernua*, etc (Prach et al., 2012). Microbial communities are frequently observed in non-vegetated areas and they can form biological soil crusts (BSCs) on substrates ranging from fragmented rocks, gravels, and soils (Belnap and Lange, 2003). This type of community can be found in almost any terrestrial environment where vegetation does not cover 100% of the soil surface. The BSCs include various combinations of microphytic communities including microbial phototrophs (cyanobacteria and eukaryotic microalgae), heterotrophic bacteria, fungi, mosses, and lichens (Belnap and Lange, 2003; Büdel and Colesie, 2014). BSCs are involved in many important processes of the soil ecosystems such as nitrogen fixation, moisture trapping, stabilization of soil and organic carbon sequestration (Langhans et al., 2009; Huang et al., 2014; Stewart et al., 2014). In partially vegetated areas, BSCs also constitute a unique environment which influences the growth of vascular plants and abundance and structure of small animal communities (Bowker et al., 2010; Langhans et al., 2010).

BSCs in Polar Regions develop in a similar way as in warmer environments (Belnap and Lange, 2003; Pushkareva et al., 2015; Pessi et al., 2018) and diverse BSC types were described in Polar Regions (Williams et al., 2016). In summary, first, the pioneer organisms, mainly cyanobacteria, colonize the soil surface. Then, algae, mosses and lichens gradually appear, resulting in a better developed BSC containing higher nutrients concentrations. Further, the BSC community gradually develops through a succession of different organisms assemblages till it reaches a climax stage with a stable community where the lichens, mosses or microbial phototrophs will no longer change unless another disruption occurs (Büdel and Colesie, 2014). Filamentous cyanobacteria such as *Leptolyngbya*, *Phormidium,* and *Microcoleus* have a pivotal role in BSC formation due to the production of extracellular polymeric substances (EPS), which promote the stabilization of the soil surface, moisture retention, and protection against erosion (Hu et al., 2012; Büdel et al., 2016).

Moreover, as climate change is already happening and is even worsened in some cases by anthropogenic impact, it is important to characterize the fragile Polar ecosystems. In particular, the BSC communities that are intimately linked to harsh and arid microclimatic conditions merit particular attention. Thereby, this study aims to compare communities of soil microbial phototrophs in three types of extreme Arctic environments which differ in the duration of ice-free conditions: old vegetated soils in a raised marine terrace, BSC located at a higher altitude and a poorly-developed BSC in front of a glacier foreland. We hypothesized that soil properties and microclimatic conditions greatly influence the biovolume and diversity of the soil microbial phototrophs. Furthermore, the sampling sites serve as control areas for open top chambers (OTCs) which were placed in 2011. Hence, the present assessment constitutes the baseline data for later comparisons with samples collected from inside the OTCs in future, which will provide insights on the effects of mimicked climate change on Arctic soil micro-phototrophic communities.

METHODS

Site Description

Sampling sites were located in the vicinity of Petunia Bay, the north branch of Billefjorden, Central Svalbard (**Figure 1**). The mean annual air temperatures in the study area range from -−3.7°C at the sea level to <-8.4°C (top of Mumien Peak) in higher-elevation sites (Ambrožová and Láska, 2017). The coastal zone of Petunia Bay has a short summer (from June to September) with the mean monthly air temperatures above 0°C reaching maximum at 15°C in July (Láska et al., 2012). Annual precipitation is about 200 mm.

Three different localities were chosen for the study: a raised marine terrace (Site T), the Mumien Peak (Site P), and the foreland of the Hørbye Glacier (Site G). Site T with stable old soils uplifted above the sea level was fully covered by plants, including vascular plants such as *Polygonum viviparum*, *Salix polaris*, *Carex rupestris*, and *Dryas octopetala* (Prach et al., 2012). Site P located at the altitude of 442 m a.s.l. had mid-developed BSC with presence of mosses and lichens. The BSC were growing on weathered sedimentary rocks on unstable terrain slopes (average slope of 27°). Finally, BSC from the recently deglaciated Site G was poorly-developed without any presence of lichens, mosses, and vascular plants. Summary of the geographical characteristics of the sampling sites and geological characteristics of the soils (Szczucinski and Rachlewicz, 2007; Hanáček et al., 2013) are presented in **Table 1**.

Soil Microclimate Measurements

The soil temperature (ST) and soil water content (SWC) were measured using three automatic meteorological stations (EMS Brno, CZ). The ST was monitored by a Pt100/Class A platinum resistance thermometer (EMS Brno, CZ) with an accuracy of ± 0.15°C. The SWC monitoring based on the soil capacitance method was conducted using a Decagon 10HS soil moisture sensor (Decagon, USA) with an accuracy of ±2%, given by a calibration equation for the mineral soil. All sensors were placed in the shallow profile at 5 cm depth and data were recorded at 60-min intervals with an EdgeBox V8 multichannel datalogger (EMS Brno, CZ). The measurements were carried out in the period from 1 July 2010 to 31 July 2014, but for evaluation of soil thermal regime and water availability, only full summer seasons, defined according to the thawing period (French, 2007) when the soil temperature was higher than 0°C, were chosen. The microclimatic parameters served as the basis for the calculation of the length of the thawing period at each site, mean and extreme ST and SWC during the thawing period in 2011, 2012, and 2013. Obtained data were analyzed by the Mini32 software (EMS Brno, CZ) with subsequent processing using Statistica® software.

Sampling Procedure

Top soils or BSCs with a depth of 2–3 cm were collected from each site in August of 2011, 2012, and 2013. Three replicates were sampled within each site ∼5 m apart from each other and each replicate contained three soil cores (5.6 cm in diameter) mixed together. Soils from site T were fully covered by plants when collected and, subsequently, plants were removed from the

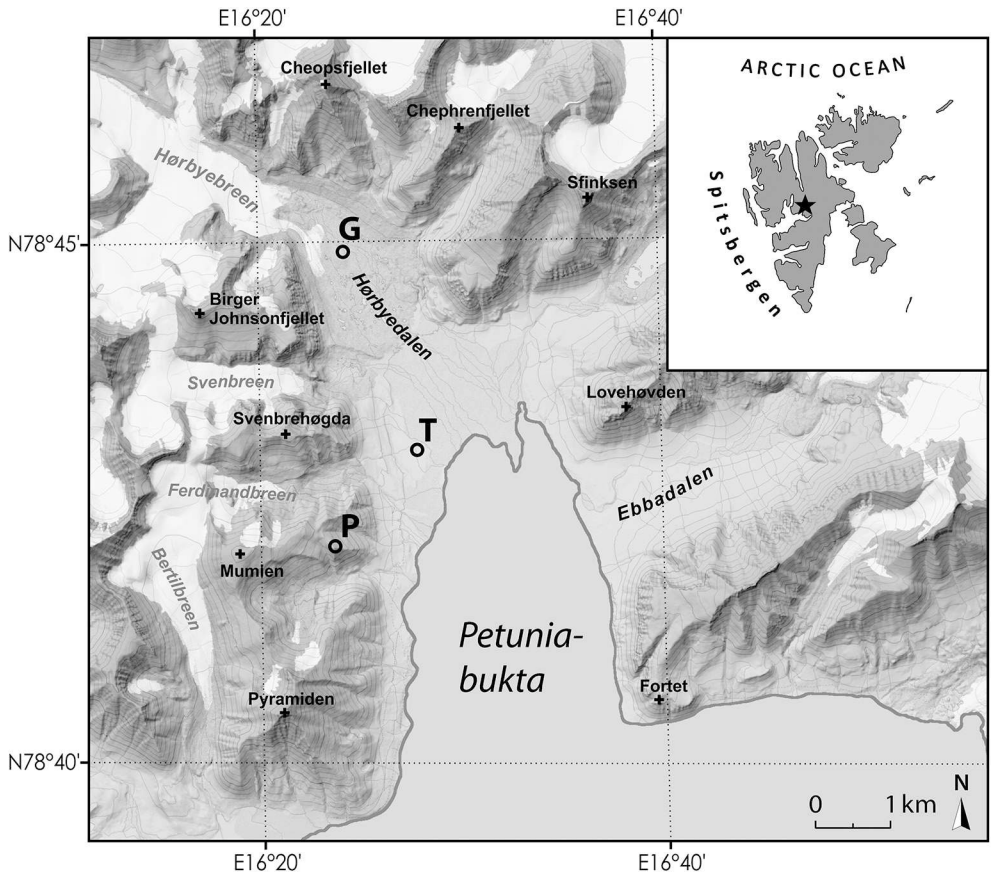

FIGURE 1 | Map of Petunia Bay and the sampling sites: T, P, and G. The modified map of Petunia Bay is based on the Svalbardkartet data, Norwegian Polar Institute.

samples. Collected samples were placed into zip bags, transported to the laboratory in dry ice and kept frozen at −20°C for further analyses.

Soil Physicochemical Analysis

Soil samples were passed through a sieve (2 mm mesh) and chemical analysis was performed according to Czech and European Union standards (ISO 10390, ISO 10523, ČSN EN 27888, ISO 11465, ČSN EN ISO 11732, ČSN EN ISO 13395, and ČSN EN ISO 15681–1). Conductivity was evaluated in demineralized water. Soil pH was measured in 1 M KCl. The percentage of total organic carbon (TOC) was determined by wet oxidation with acidified dichromate. NH_4-N and NO_3-N concentrations were measured using a QuikChem® 8500FIAAutomated Ion Analyzer (Lachat Instruments, Loveland, USA). Phosphorus was detected as PO_4-P using ascorbic acid–molybdate and a SHIMADZU UV-1650PC spectrophotometer. Macroelements (Ca, Mg, K, Na) were analyzed using a ContrAA® atomic absorption spectrometer (Analytik Jena, Jena, Germany).

Biovolume of Cyanobacteria and Eukaryotic Microalgae

Cyanobacterial cell biovolume was estimated using light and epifluorescence microscopy (Kaštovská et al., 2005) as described in Pushkareva et al. (2017) for the samples collected in 2011 and 2012. Three groups of cyanobacteria were determined according to their cell morphology: unicellular, filamentous, and heterocystous cyanobacteria. Moreover, diatoms and coccoid microalgae (green algae Chlorophyta and yellow-green algae Xanthophyceae) were also distinguished within the eukaryotic microalgae. Filamentous eukaryotic microalgae were not observed in the samples. Basic geometric equations for cylinders

TABLE 1 | The geographical and geological characteristics of the sampling sites in Petunia Bay, Central Svalbard.

	Site T	Site P	Site G
Latitude (° N)	78.71050	78.70116	78.74818
Longitude (° E)	16.46101	16.39016	16.40096
Altitude (m a.s.l.)	16	442	73
Slope (°)	< 2	27	<2
Aspect (°)	–	54	–
Geological characteristics*	Carboniferous-Permian sedimentary rocks	Devonian sedimentary rocks	Quaternary covers
Type of surface	Raised terrace, tundra vegetation	Rock outcrops, regolith material	Ice-cored moraine, debris material

*Szczucinski and Rachlewicz (2007), Hanáček et al. (2013).

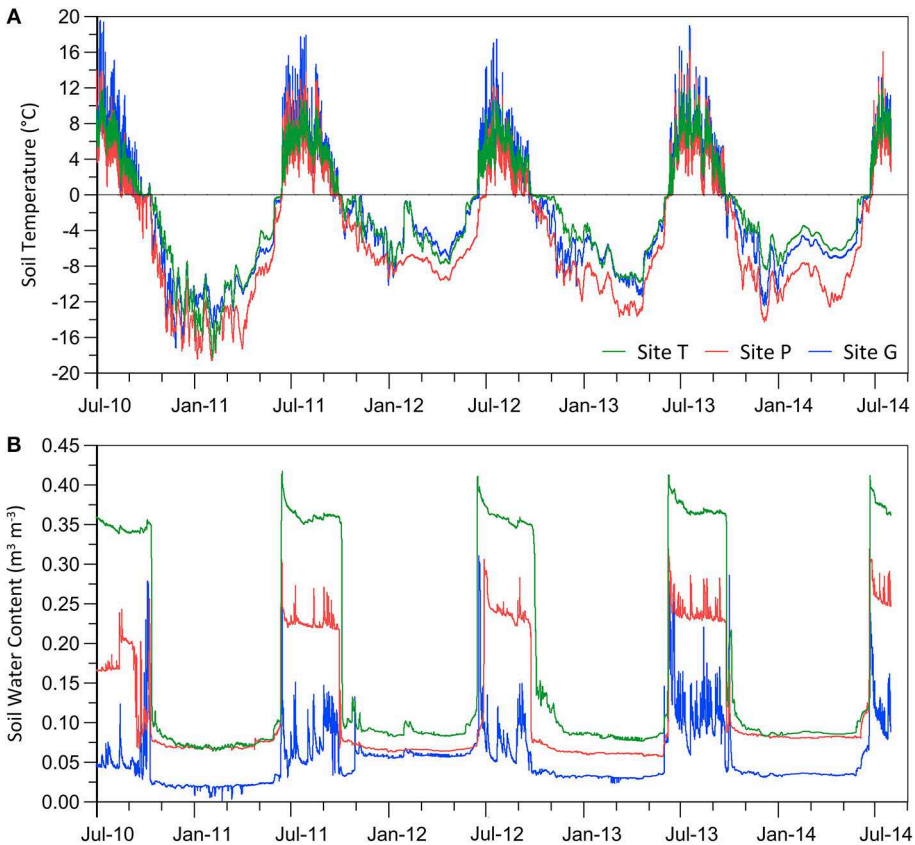

FIGURE 2 | Soil temperature **(A)** and soil water content **(B)** in the three studied sites in the period of 2010–2014.

with hemispherical ends and spheres were applied to calculate the biovolume ($\mu m^3\ g^{-1}$) of soil samples (Hillebrand et al., 1999).

Cyanobacterial Diversity by a Culture-Independent Approach

To extract DNA from the nine samples collected in 2012, two DNA Isolation Kits (MOBIO, Carlsbad, CA, USA) were tested, the PowerBiofilm and PowerSoil. Both kits resulted in similar DNA yields and, therefore, the PowerBiofilm DNA Isolation Kit was used according to the manufacturer's instructions.

The V3-V4 region of the 16S rRNA gene was amplified using the cyanobacteria-specific primers 359F and 781Ra/781Rb (Nübel et al., 1997) in separate reactions for each reverse primer (Boutte et al., 2006). Barcode sequences had been added to both forward and reverse primers and were specific to each sample to enable multiplexing. PCR reactions in triplicate for each set of primers were performed as described in Pessi et al. (2016). Triplicates were later pooled together, purified using the GeneJet PCR Purification Kit (Thermo Scientific, Waltham, MA, USA) and quantified using the Quant-iTPicoGreen dsDNA Assay Kit (Life Technologies, Carlsbad, CA, USA). Libraries were pooled in equimolar concentrations and sent to GENEWIZ (South Plainfield, NJ, USA) where sequencing adaptors were ligated to the amplicons and paired-end sequences (2 × 300 bp) were obtained using the Illumina MiSeq v3 platform. Sequencing of three soil samples (one sample from Site T and two samples from Site G) was not successful, and thus these samples were excluded from further analyses. Raw sequences were deposited in Sequence Read Archive (SRA) with accession number PRJNA561039.

Quality filtering of obtained sequences and their clustering into operational taxonomic units (OTUs) were performed using UPARSE at 97% sequence identity (Edgar, 2013) according to Pessi et al. (2016). Reads shorter than 370 bp and with more than 0.5 expected errors were discarded. OTUs were classified using CREST [30] based on the Greengenes database (McDonald et al., 2012). Non-cyanobacterial OTUs were discarded and the most closely related isolate for each OTU was obtained using the SeqMatch tool from RDP (Cole et al., 2014).

Rarefaction curve (**Supplementary Figure 1**) and alpha diversity indices (**Supplementary Table 1**) were calculated using QIIME 1 (Caporaso et al., 2010) after normalizing the number of sequences to those of the samples with the lowest number of sequence reads (1983 sequences). Beta diversity was calculated using the UniFrac distance metric (Lozupone and Knight, 2005) with Weighted Paired Group Method of Averaging (WPGMA).

Diversity of Eukaryotic Microalgae by a Culture-Dependent Approach

Soil samples collected in 2012 were pre-cultivated on plates with solid BG11 medium (Stanier et al., 1979). To obtain unialgal strains, the dilution plate method was used (Elster et al., 1999). The isolated strains of eukaryotic microalgae were kept in an illuminated incubator at a temperature of 6°C and continuous lighting with a photon flux density of 20 μmol m^{-2} s^{-1}.

Genomic DNA of microalgal strains was extracted using the XS method (Tillett and Neilan, 2000) according to Yilmaz et al. (2009). The 18S rRNA gene was amplified using primers NS1 (White et al., 1990) and 1650R vivi (Kipp, 2004) as follows: an initial denaturation step at 94°C for 5 min, followed by 35 cycles of DNA denaturation at 94°C for 30 s, primer annealing at 55°C for 54 s, strand extension at 72°C for 2 min, and a final extension step at 72°C for 10 min. PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN, The Netherlands). Sequencing was carried out by the Laboratory of Genomics, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice with the ABI PRISM 3130 XL sequence analyzer (Applied Biosystems, Life Technologies Corp., CA, USA) using primers NS1 and 1150R (White et al., 1990). Obtained sequences were analyzed using BioEdit v7.2.5 (Hall, 1999) and deposited in GenBank with accession numbers MK929233-MK929255. The most closely related isolates were fetched from GenBank using BLAST. All sequences were aligned using MAFFT (Katoh and Standley, 2013). A phylogenetic tree comprising the algal isolate sequences and their best GenBank hits was constructed using the maximum likelihood method based on the Jukes-Cantor model in MEGA6 (Tamura et al., 2013).

Statistical Analysis

The statistical analyses were performed in the statistical software JMP 14.0.1. Normality of variance was assessed using Shapiro–Wilk's test and, if necessary, data were Box Cox or Square Root transformed. Differences in soil chemical parameters were evaluated using the one-way ANOVA and Tukey's HSD *post-hoc* test. Two-way ANOVA was used to test the effects of the factors (site and year) on soil chemistry and biovolume. The relationships between biovolume of microbial phototrophs and soil parameters were modeled using Pearson correlation coefficient (PCC).

RESULTS

Microclimatic Parameters

Soil temperature (ST) and soil water content (SWC), measured at 5 cm depth during the three full summer seasons, showed differences between the three studied sites (**Figure 2**; **Supplementary Table 2**). The thawing period (when the ST was above 0°C) lasted from June till September and varied from 72 to 107 days, depending on the site and year (**Supplementary Table 2a**). The shortest thawing period (72–99 days) in the studied sites was recorded in 2012. Year-to-year differences in the duration of the thawing period varied between 8 and 9 days at the low-lying Sites T and G, but reached 25 days

TABLE 2 | Soil chemistry in the three studied localities with standard error (SE) measured in the samples collected in 2011, 2012, and 2013.

	pH (SE)		Conductivity, µS/cm (SE)		$N\text{-}NH_4$, mg kg^{-1} (SE)		$N\text{-}NO_3$, mg kg^{-1} (SE)		$P\text{-}PO_4$, mg kg^{-1} (SE)		Organic C, % (SE)		Ca, mg kg^{-1} (SE)		Mg, mg kg^{-1} (SE)		K, mg kg^{-1} (SE)		Na, mg kg^{-1} (SE)	
Site T																				
2011	$7.7^{B,a}$	(<0.1)	$114.4^{B,a}$	(22.2)	$5.5^{A,b}$	(2.0)	$0.4^{A,b}$	(0.2)	$0.5^{A,c}$	(<0.1)	$12.5^{A,a}$	(1.4)	$5170^{A,b}$	(235)	$238^{B,b}$	(4)	$64.7^{A,b}$	(6.1)	$20.5^{A,a}$	(10.4)
2012	$7.7^{B,a}$	(<0.1)	$146.3^{A,a}$	(6.1)	$15.5^{A,a}$	(5.0)	$1.7^{B,a}$	(1.2)	$5.1^{A,b}$	(0.8)	$13.6^{A,a}$	(1.0)	7962	NA	808	NA	108.4	NA	21.1	NA
2013	$7.4^{B,b}$	(<0.1)	$155.8^{A,a}$	(2.1)	$6.4^{A,ab}$	(0.9)	$0.3^{A,b}$	(<0.1)	$17.6^{A,a}$	(0.9)	$14.6^{A,a}$	(1.5)	$7690^{A,a}$	(219)	$382^{B,a}$	(14)	$334.4^{A,a}$	(49.1)	$19.3^{A,a}$	(1.5)
Site P																				
2011	$8.5^{A,a}$	(0.1)	$96.0^{B,a}$	(6.0)	$4.0^{A,a}$	(2.9)	$0.7^{A,b}$	(0.3)	$0.1^{B,c}$	(<0.1)	$5.1^{B,a}$	(0.6)	$4186^{B,a}$	(526)	$818^{A,b}$	(127)	$31.7^{C,b}$	(5.7)	$12.4^{A,a}$	(6.1)
2012	$8.5^{A,a}$	(<0.1)	$93.7^{B,a}$	(11.3)	$3.4^{B,a}$	(0.8)	$21.5^{A,a}$	(13.8)	$5.9^{A,b}$	(0.8)	$3.6^{B,b}$	(0.3)	3493	NA	1476	NA	20.7	NA	4.4	NA
2013	$8.3^{A,a}$	(0.1)	$85.5^{B,a}$	(4.3)	$1.7^{B,a}$	(0.2)	$0.4^{A,b}$	(<0.1)	$20.6^{A,a}$	(1.9)	$3.6^{B,b}$	(0.6)	$5156^{B,a}$	(615)	$1806^{A,a}$	(291)	$94.8^{B,a}$	(18.5)	$17.3^{A,a}$	(2.7)
Site G																				
2011	$8.4^{A,a}$	(<0.1)	$432.3^{A,a}$	(129.3)	$2.4^{A,a}$	(0.9)	$0.2^{B,a}$	(<0.1)	$0.1^{B,c}$	(<0.1)	$3.4^{C,a}$	(<0.1)	$5042^{AB,b}$	(344)	$176^{B,b}$	(6)	$48.5^{B,b}$	(2.1)	$1.3^{B,b}$	(1.7)
2012	$8.4^{A,a}$	(0.2)	$88.1^{B,b}$	(5.5)	$1.6^{C,a}$	(0.2)	$0.2^{C,a}$	(<0.1)	$2.6^{B,b}$	(0.3)	$3.0^{B,a}$	(<0.1)	5293	NA	323	NA	57.0	NA	5.2	NA
2013	$8.3^{A,a}$	(<0.1)	$73.0^{C,c}$	(2.1)	$0.8^{C,b}$	(<0.1)	$0.1^{B,a}$	(<0.1)	$16.8^{A,a}$	(1.9)	$2.4^{C,b}$	(0.3)	$7172^{A,a}$	(156)	$345^{B,a}$	(3)	$101.9^{B,a}$	(4.1)	$20.6^{A,a}$	(3.7)
Two-Way ANOVA (F Ratios)																				
Site	232.91 ***		29.38***		74.01***		66.03***		38.00***		544.83***		28.95***		310.15***		99.79***		6.93*	
Year	16.47 ***		20.80***		19.29***		23.69***		2114.56***		5.43*		83.68***		114.49***		364.44***		14.55**	
Site x Year	ns		31.15***		6.43**		ns		17.21***		6.87**		4.62*		9.35**		28.61***		8.89**	

Same letters indicate no statistical difference between groups according to one-way ANOVA followed by Tukey's pairwise posthoc tests ($p < 0.05$): (A–C), between sites at the same year; (a–c), between years within each site. Two-way ANOVA results show F ratios. For Ca, Mg, K, and Na, two-way ANOVA was done only with samples from 2011 and 2013.

****$p < 0.001$, **$p < 0.01$, *$p < 0.05$, ns, non-significant.*

NA means that three soil samples were mixed together prior measurements and therefore SD is not available.

at the highest elevated Site P between 2012 and 2013. During the studied thawing periods, July was the warmest month in the three sites and the highest mean ST was recorded at the Site G (8.5°C in July) (**Supplementary Table 2b**). The lowest mean ST in the thawing period was reported in June at the Site P, reaching only 0.7°C.

The highest SWCs among the studied sites were recorded during the thawing period of 2013, wherein the mean SWC varied from 0.12 to 0.37 $m^3\ m^{-3}$ (**Supplementary Table 2a**). The maximum saturation level given by the highest SWC (up to 0.42 $m^3\ m^{-3}$ at the Site T) was reached at the beginning of the thawing period in the second half of June, only a few days after the final snow melting (**Supplementary Table 2b**). The minimum SWC (0.03 $m^3\ m^{-3}$) occurred in Site G at the beginning of the thawing season.

Large seasonal and day-to-day variations in both ST and SWC were observed during each thawing period (**Figure 2**). In contrast to other sites, a higher variability of the soil thermal regime was observed at the Site G, where the lowest level of SWC was also measured. Moreover, a high variation in ST was found at the higher-elevation Site P composed of weathered sedimentary rocks on unstable terrain slopes (average slope of 27°) without any presence of vegetation (see **Table 1**) though the SWC values appear very stable during the same period.

Soil Properties

Chemical parameters of the soil crusts and vegetated soil samples measured during three summers varied significantly between the sites and years (**Table 2**). The pH fluctuated between 7.4 and 8.5 across the studied localities and was significantly lower at Site T than in other sites. On the other hand, conductivity, TOC and NH_4-N contents were high at Site T. Low nutrient concentrations were recorded in the poorly developed BSC from the glacier foreland (Site G). The ammonium concentration (NH_4-N) was higher than the nitrate (NO_3-N) concentration at all sites and ranged from 0.8 to 15.5 mg kg^{-1} soil while the nitrate concentrations were negligible, except for the soil collected in 2012 at Site P (21.5 mg kg^{-1} soil).

Biovolume of Microbial Phototrophs

Total cyanobacterial biovolume was the highest in Site P with average values of 76.8 × 10^6 and 39.7 × 10^6 $\mu m^3\ g^{-1}$ for the samples collected in 2011 and 2012, respectively (**Figure 3**; **Supplementary Table 3**). Lower cyanobacterial biovolumes were observed in Site G (average of 12.5 and 9.9 × 10^6 $\mu m^3\ g^{-1}$ in 2011 and 2012, respectively) and Site T (average of 17.6 and 3.9 × 10^6 $\mu m^3\ g^{-1}$ in 2011 and 2012, respectively). Filamentous cyanobacteria were the dominant cyanobacteria in all studied sites and constituted a major fraction within each locality (29–73% of total biovolume). In addition, they had higher biovolume in site P than in the other sites (average of 39.0 and 25.6 × 10^6 $\mu m^3\ g^{-1}$ in 2011 and 2012, respectively) together with heterocystous (average of 23.2 and 6.8 × 10^6 $\mu m^3\ g^{-1}$ in 2011 and 2012, respectively) and unicellular cyanobacteria (average of 14.6 and 7.4 × 10^6 $\mu m^3\ g^{-1}$ in 2011 and 2012, respectively).

Eukaryotic coccoid microalgae together with diatoms had higher biovolume in the samples collected in 2011 than in those from 2012 (**Figure 3**; **Supplementary Table 3**). The highest biovolume of eukaryotic coccoid microalgae was recorded in Site T (average of 19.4 and 2.9 in 2011 and 2012, respectively). In contrast, their lowest biovolume (average of 0.6 and 0.2 × 10^6 $\mu m^3\ g^{-1}$ in 2011 and 2012, respectively) was observed at Site G where diatoms were present only in one sample collected in 2011. In addition, diatoms were very rare, but slightly more abundant in Site P than in the other two sites.

Diversity of Microbial Phototrophs

A total of 141132 quality reads were obtained for six samples using amplicon sequencing of the 16S rRNA gene. After bioinformatic analysis, 69631 cyanobacterial sequences remained and were later grouped into 137 OTUs. The majority of the OTUs belonged to the order Synechococcales (78 OTUs) and the rest of the OTUs were from the orders Chroococcales (19 OTUs), Oscillatoriales (12 OTUs), Nostocales (11 OTUs), and Gloeobacterales (10 OTUs) (**Figure 4**). In addition, 7 OTUs were not classified further than phylum.

To compare cyanobacterial community composition between the study sites, the dataset was normalized to 1983 sequences per sample. After the normalization, 128 OTUs remained and an average of 92, 69, and 67 OTUs were observed in Sites P, T, and G, respectively (**Figure 4A**; **Supplementary Table 1**). Good's coverage estimator was 98–99%, indicating that the large majority of the cyanobacterial diversity was captured in the analysis (**Supplementary Table 1**). A total of 52 OTUs were shared between the three studied localities (**Supplementary Figure 2**). Furthermore, 27 OTUs were unique to Site P while 3 OTUs were only found in the Sites T and G. The three localities were dominated by cyanobacteria from the order Synechococcales (84.4–88.2% of all sequences), where the genus *Leptolyngbya* constituted a major fraction (52.6–74.0% of all sequences) and was the most abundant in Site G (**Figure 4B**). Filamentous cyanobacteria from the order Oscillatoriales ranged from 7.26 to 7.82% of all sequences in each site. Furthermore, Chroococcales (1.36–3.31% of all sequences), Gloeobacterales (1.16–2.22% of all sequences), and Nostocales (1.55–1.97 % of all sequences) were also present in the three studied sites. OTU 1 (100% similar to *Phormidium* sp. CYN64) was the most abundant in Site T, whereas OTU 2 (99.5% similar to *Leptolyngbya antarctica* ANT.L67.1) was dominant in Site P and quite abundant in the other sites (**Table 3**). Additionally, OTU 6 (100% similar to *Leptolyngbya frigida* ANT.L52B.3) dominated in Site G. These three hits were filamentous cyanobacteria collected in Antarctic lakes and ponds (Taton et al., 2006; Martineau et al., 2013).

WPGMA analysis showed that the cyanobacterial community compositions in the three localities differed from each other (**Supplementary Figure 3**). Moreover, the three replicates from Site P formed a well-defined cluster based on the obtained cyanobacterial OTUs, while the samples from Sites T and G clustered erratically.

A total of 23 isolates of eukaryotic microalgae including Chlorophyte (15 isolates) and Xanthophyceae (8 isolates) was obtained from the three localities (**Table 4**; **Supplementary Figure 4**). The majority of the eukaryotic microalgae (13 isolates) were isolated from Site T, while 6 and 4 isolates were obtained from Sites G and P, respectively. Moreover,

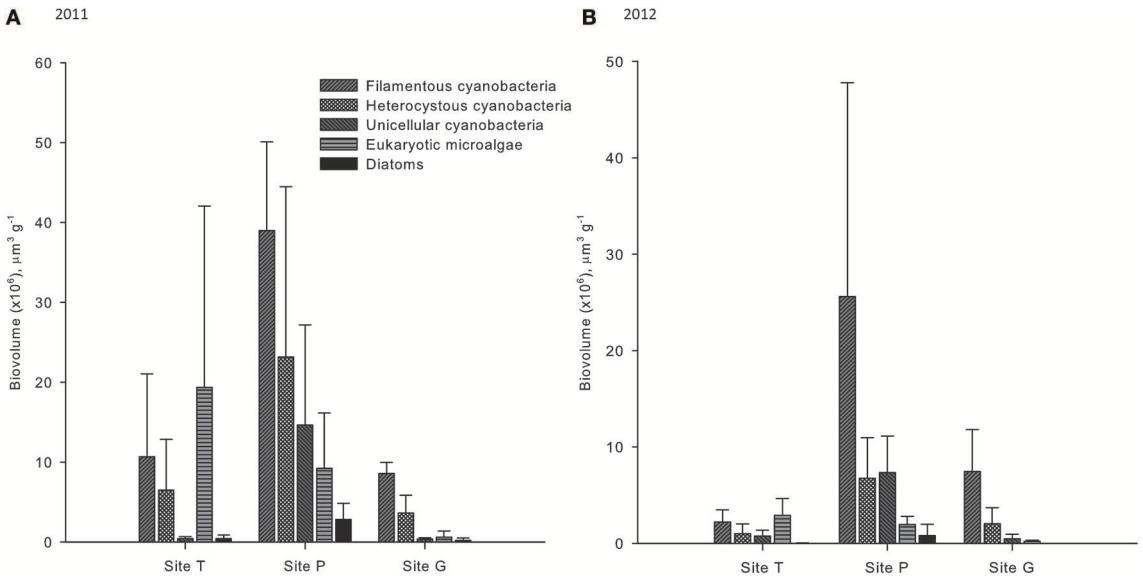

FIGURE 3 | Biovolume of microbial phototrophs per 1 g of dry soil in the samples collected in 2011 **(A)** and 2012 **(B)**.

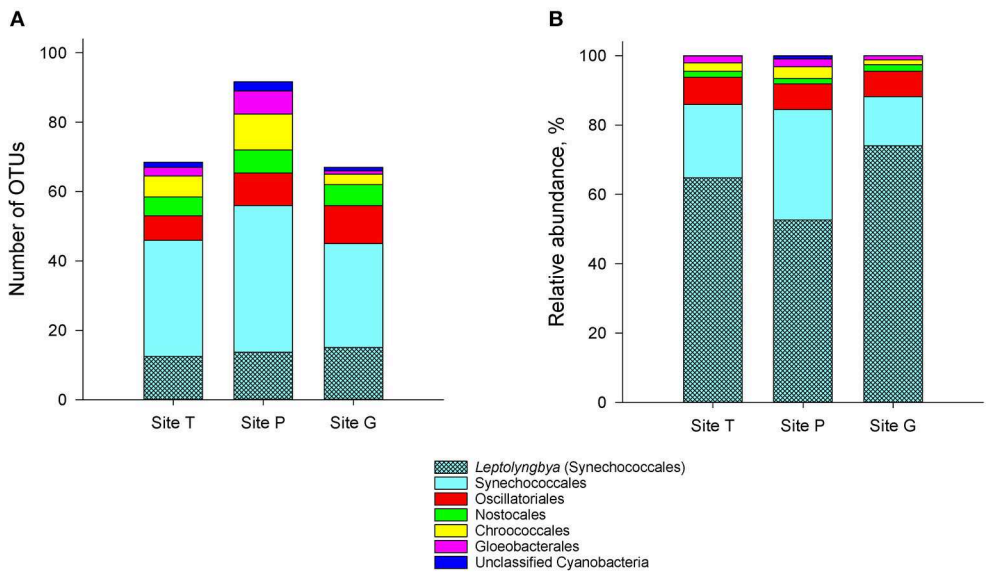

FIGURE 4 | Cyanobacterial community composition in three studied localities based on Illumina sequencing of the 16S rRNA gene. Data were calculated after the normalization to 1983 sequences per sample. **(A)** Richness expressed as the number of OTUs and **(B)** distribution of the reads expressed as relative abundance.

no yellow-green algae were isolated from Site P. Obtained microalgal sequences (except from isolates 4, 5, and 7) were more than 99 % similar to the sequences from NCBI database. Isolates 1 and 6 (*Chloromonas*-affiliated) as well as isolates 8, 9, 11, and 13 (*Coccomyxa*-affiliated) formed groups of related strains isolated from two or three different sites (**Supplementary Figure 4**). In contrast, strains related to *Koliella/Stichococcus* were only isolated from site T. Xanthophyceae-affiliated strains related to *Heterococcus* and *Xanthonema* were isolated from sites T and G.

Relationship Between Biovolume of Microbial Phototrophs and Soil Chemistry

Pearson correlation coefficient showed a positive effect of pH and conductivity on biovolume of filamentous cyanobacteria ($p < 0.05$; **Table 5**). On the other hand, there were negative correlations ($p < 0.05$) between macroelements contents (Ca and K) and cyanobacterial biovolumes. Furthermore, biovolume of eukaryotic microalgae was positively correlated ($p < 0.05$) to NH_4-N, TOC, and Na, while diatoms were so rare that correlations were not possible to calculate.

TABLE 3 | The most abundant* cyanobacterial operational taxonomic units (OTUs) in the three sites.

OTU	Relative abundance**, %			Best SeqMatch isolate hit (Accession number, ID %)
	Site T	Site P	Site G	
OTU2	17.10	**22.64**	13.26	*Leptolyngbya antarctica* ANT.L67.1 (AY493572, 99.5%)
OTU1	**32.17**	3.68	8.32	*Phormidium* sp. CYN64 (JQ687330, 100%)
OTU6	2.24	5.56	**27.69**	*Leptolyngbya frigida* ANT.L52B.3 (AY493612, 100%)
OTU11***	0.71	0.49	16.29	*Phormidesmis priestleyi* ANT.LG2.4 (AY493580.1, 100%)
OTU4***	0.40	12.12	0.15	*Leptolyngbya* sp. LLi18 (DQ786166, 97.5%)
OTU10	5.82	3.26	2.72	*Synechococcus* sp. PS715 (AF216952, 99.7%)
OTU7***	0.76	8.02	0.10	*Calothrix* sp. KVSF5 (EU022730, 95.1%)
OTU5	3.48	2.56	2.02	*Leptolyngbya* sp. ANT.L52.1 (AY493584, 97.5%)
OTU3	5.72	1.28	1.01	*Arthronema africanum* SAG 12.89 (AB115966, 93.1%)
OTU8	2.45	2.32	2.37	*Microcoleus rushforthii* UTCC 296 (AF218377, 100%)
OTU189	3.66	0.44	2.02	*Phormidium* sp. CYN64 (JQ687330, 96.4%)
OTU15***	1.69	3.11	0.81	*Leptolyngbya nostocorum* UAM 387 (JQ070063, 97.8%)
OTU12***	0.13	4.91	0.20	*Calothrix* sp. KVSF5 (EU022730, 94.5%)
OTU315	0.40	0.84	3.88	*Arthronema africanum* SAG 12.89 (AB115966, 93.4%)

OTUs are listed in decreasing order based on their total relative abundance.
**OTUs with more than 100 sequences in sum for six samples after the data normalization to 1983 sequences.*
***Relative abundances obtained after data normalization to 1983 sequences.*
****OTUs with 100% similarity to sequences of cyanobacterial crusts in Petunia Bay (Pushkareva et al., 2015).*
Bold values correspond to the highest abundance within each site.

DISCUSSION

Microbial Phototrophs Inhabiting Arctic Soil Environment

The morphological and molecular methods revealed conspicuous differences in the phototrophic community composition in the three studied localities. Site P had the highest biovolume of microbial phototrophs and cyanobacterial OTU richness as well as the highest number of unique OTUs in comparison to the other sites (**Figures 3**, **4A**; **Supplementary Figure 2**). Frequent occurrence of snowfall events and the consequent fast snowmelt during summer months at high altitude provided the liquid water favoring the seasonal activity of soil microbial phototrophs (Janatková et al., 2013; Pushkareva and Elster, 2013). It could also be explained by the soil organic matter accumulated in the mucilaginous BSC, which is subjected to little turn-over due to the limited period of time where metabolic activity is possible (about 3 months). Thus, the creation of a kind of "cocoon" could mitigate the harsh conditions of a weathered rocky substrate on a slope. Though the BSC from glacier foreland Site G was also dominated by cyanobacteria, a much lower biovolume and OTU richness were observed, probably, as a consequence of frequent mechanical disturbances (cryoturbation, water erosion, etc.) connected with the low availability of mineral nutrients and organic carbon, which are the key parameters in shaping BSC communities (Housman et al., 2006; Newsham et al., 2010; Pietrasiak et al., 2013; Pushkareva et al., 2015).

Samples from Sites P and G were dominated by cyanobacteria, where filamentous taxa constituted a major fraction (**Figures 3**, **4**) and, furthermore, sequences affiliated to *Leptolyngbya* spp. were prevalent (**Table 3**). Besides, OTU 1 (100% similar to *Phormidium* sp. CYN64) was the dominant cyanobacterial OTU in the Site T and similar sequences were also found to prevail in the lichenized BSC in the coastal zone of Petunia Bay in Svalbard (Pushkareva et al., 2015). Indeed, filamentous cyanobacteria usually dominate polar terrestrial ecosystem due to their motility and mucilage production (Kaštovská et al., 2005; Pushkareva et al., 2016; Rippin et al., 2018). Heterocystous cyanobacteria (Nostocales), which are an important source of fixed nitrogen in Arctic soils, had a higher biovolume in Site P than in the other sites. It has been shown that heterocystous cyanobacteria increase in quantity with elevation, because their biovolume is independent of the concentration of organic matter, unlike filamentous cyanobacteria (Řeháková et al., 2011). Moreover, at the OTU level, the majority of Nostocales sequences were similar to *Calothrix* sp. (similarity 94–95%) and sequences affiliated to this genus represented 13% of the reads in Site P. In addition, they were identical to sequences of the "OTU4" previously found in two BSCs in the nearby coastal zone of Petunia Bay (Svalbard), one from poorly developed cyanobacterial crust and one from a mid-developed with a mixture of lichens and cyanobacteria, showing evidence of a local distribution of this phylotype (Pushkareva et al., 2015). This sequence represents a new phylotype that has not yet been cultivated and thus, its precise identity is still elusive. Similarly, biovolume of unicellular cyanobacteria was higher in Site P than in other localities, possibly because they do not require a stable substrate and high organic matter content (Kaštovská et al., 2005; Řeháková et al., 2011).

In contrast, the nutrient-rich vegetated soils from Site T had a lower cyanobacterial biovolume than in Site P as a result of light deficiency, caused by overshadowing due to the dense presence

TABLE 4 | Eukaryotic microalgae isolated from the three localities.

Microalgal isolate	Site	Most closely related GenBank isolate sequence (Accession number, ID %)
Chlorophyte (Green Algae)		
Isolate 1	P	*Chloromonas rosae* SAG51.72 (AB624565.1, 99%)
Isolate 2	T	*Koliella antarctica* SAG 2030 (AJ311569.1, 99%)
Isolate 3	T	*Chlamydomonas* sp. ISBAL (JN903985.1, 99%)
Isolate 4	T	*Pseudomuriella aurantiaca* SAG 249-1 (X91268.1, 92%)
Isolate 5	G	*Chloromonas macrostellata* SAG 72.81 (JN903986.1, 98%)
Isolate 6	T	*Chloromonas rosae* SAG51.72 (AB624565.1, 99%)
Isolate 7	P	*Bracteacoccus occidentalis* ACSSI 221 (MG582203.1, 95%)
Isolate 8	P	*Coccomyxa* sp. KGU-D001 (AB742451.1, 99%)
Isolate 9	G	*Coccomyxa* sp. KGU-D001 (AB742451.1, 99%)
Isolate 10	P	*Mychonastes zofingiensis* CCAP 211/14 (GU827478.1, 100%)
Isolate 11	G	*Coccomyxa subellipsoidea* CCAP 812/3 (HG972972.1, 100%)
Isolate 12	T	*Stichococcus* sp. SAG 2482 (KP081395.1, 99%)
Isolate 13	T	*Coccomyxa subellipsoidea* CCAP 812/3 (HG972972.1, 100%)
Isolate 14	T	*Stichococcus* sp. WB47 (KF144240.1, 99%)
Isolate 15	T	*Stichococcus jenerensis* KP09HW3001 (KX094816.1, 99%)
Xanthophyceae (Yellow-Green Algae)		
Isolate 16	T	*Heterococcus chodatii* SAG 835-3 (AM490822.1, 100%)
Isolate 17	T	*Heterococcus chodatii* SAG 835-3 (AM490822.1, 99%)
Isolate 18	T	*Tribonema ulotrichoides* SAG 21.94 (AM490817.1, 99%)
Isolate 19	G	*Heterococcus caespitosus* SAG 835-2a (AM490820.1, 99%)
Isolate 20	G	*Xanthonema* sp. A14 (AM491612.1, 99%)
Isolate 21	G	*Botrydiopsis constricta* (AJ579339.1, 99%)
Isolate 22	T	*Xanthonema sessile* IBSG-V98 (AM490818.1, 99%)
Isolate 23	T	*Xanthonema* sp. A14 (AM491612.1, 99%)

TABLE 5 | Pearson correlation coefficient between the biovolume of microbial phototrophs and soil physicochemical parameters.

	Cyanobacteria			Eukaryotic microalgae
	Filamentous	Heterocystous	Unicellular	
pH	0.51*			
Conductivity	0.48*			
NH_4-N				0.50*
NO_3-N			0.60**	
PO_4-P				
Organic C				0.56*
Ca	−0.62**	−0.54*	−0.48*	
Mg				
K	−0.68**	−0.62**	−0.53*	
Na				0.58*

$^{**}p < 0.01$, $^{*}p < 0.05$.

of vascular plants (Kaštovská et al., 2007). The decrease of the cyanobacterial OTU richness and biovolume in well-developed soil crusts fully covered by lichens has been shown in Svalbard (Pushkareva et al., 2015, 2017) and similar competition but with plants was probably playing a role in the case of the vegetated soils of Site T. Furthermore, the WPGMA analysis based on sequencing results showed that the three subsamples from Site P were well-grouped by their site of origin and thus were quite homogeneous (**Supplementary Figure 3**). On the other hand, the two subsamples from Site T were not grouped, whereas the only sample from Site G shared more OTUs with site P than with site T. It might be due to the unfavorable conditions in both P and G sites. Moreover, the presence of several plant species could strongly influence the composition and activity of the rhizosphere microbiota (Philippot et al., 2013) and this could lead to the cyanobacterial community heterogeneities observed within the vegetated site T.

Eukaryotic microalgae, including Chlorophyte and Xanthophyceae, prevailed over other microbial phototrophs in Site T. Besides, the majority of algal cultures, including green algae Chlorophyceae and Trebouxiophyceae and yellow-green algae Xanthophyceae, were isolated from this site (**Table 4**). These algae are typical habitants of polar vegetated soils and BSCs (Belnap et al., 2001; Borchhardt et al., 2017). Abundance of green and yellow-green algae has been shown to decrease in high altitude where vegetation is sparse or absent (Elster et al., 1999; Čapková et al., 2016). This effect could explain why their biovolume in Site P was lower than in Site T. Similarly, the biovolume of eukaryotic microalgae was the lowest in Site G due to an unfavorable microclimate, such as the low temperature and water content in summer, which negatively influence their growth and development (Elster et al., 1999).

Environmental Parameters and Their Effect on Community Composition of Microbial Phototrophs

The impact of microclimate and microenvironment around individual organisms are very significant, especially in the high-latitude regions (Elster, 2002). For example, water and temperature fluctuations are one of the most important ecological and physiological factors in the polar regions (Elster and Benson, 2004) affecting the growth of microorganisms and the biological activity of soils (Colesie et al., 2014). In the studied sites, the mean soil temperature (ST) in the period when liquid water was available, fluctuated from 4.1 to 6.7°C. Colesie et al. (2014) suggested that temperature alone is not the major controlling environmental factor in

the Polar Regions. However, temporal changes in temperature provoke a series of adaptive responses comprising resistance and tolerance to cold, freezing, drought, desiccation, and salinity stress (Tashyreva and Elster, 2012).

The highest and most stable soil water content (SWC) was recorded in the vegetated soil from the Site T during the thawing period (**Figure 2**; **Supplementary Table 2**), and could correspond to the fact that the suction of water is usually higher within the root zone of plant cover than that in bare soil (Leung et al., 2015). On the contrary, the lowest SWC with the highest seasonal variation was registered in the poorly-developed BSC in the recently deglaciated areas (Site G). The variability of soil temperature regime among the three sites showed a close relation to the water content fluctuation and saturation level of the soils. While the largest diurnal amplitudes were found for the driest soil crust from Site G (up to 11°C), the vegetated soil at the well-saturated Site T showed a twice lower temperature amplitude (**Figure 2**). On the other hand, the diurnal temperature regime at the site P showed a much smaller amplitude than lower-lying sites, as a consequence of the altitude effect on air temperature (Ambrožová and Láska, 2017), the increased snow cover occurrence and related insulation effect on the active-layer thermal regime (Westermann et al., 2009; Hrbáček et al., 2016). In addition, the microbial communities responded to environmental drivers and changed over the years. In this study, the total biovolume of microbial phototrophs was higher in the samples collected in 2011 than in those from 2012. It could be due to the lower soil temperatures in 2012 and shorter thawing period (when the ST was above 0°C), given the negative impact of low temperatures on growth and development of soil microorganisms.

Pearson correlation coefficient showed a positive relation ($p < 0.05$) between pH and biovolume of filamentous cyanobacteria (**Table 5**), which has been already shown in several studies (e.g., Nayak and Prasanna, 2007; Pushkareva et al., 2015). Furthermore, there were negative correlations ($p < 0.05$) between macroelements, such as Ca and K, and biovolume of all cyanobacterial types. Perhaps, the binding of Ca to phosphate could limit P availability, which is important for many soil ecophysiological processes (e.g., nitrogen fixation) that require its high amount (Richardson and Simpson, 2011).

CONCLUSION

In this study, we compared the community composition of microbial phototrophs in the three localities with different soil chemistry and microclimatic conditions. The BSC located at higher altitude (442 m) hosted more diverse and abundant cyanobacterial communities while eukaryotic microalgae prevailed in the vegetated soil with a more stable soil water content and temperature. Vegetation cover might be the limiting factor for the community of microbial phototrophs due to the competition and light shading. On the other hand, poorly developed BSC in the glacier foreland with low soil water content and nutrient concentrations harbored less diverse and abundant communities of microbial phototrophs than in other studied localities. Furthermore, our findings indicated a negative relationship between macroelements (Ca and K) and cyanobacterial biovolume, while a higher pH corresponded to a higher abundance of the filamentous cyanobacteria. The succession from phototrophic microbial communities dominated by cyanobacteria in harsh environmental conditions to a dominance by microalgae (green and yellow-green algae) in vegetated soils could also be the future trajectory of these High Arctic communities if higher temperatures in future are coupled with sufficient precipitations to keep a stable humidity. Therefore, our results could contribute to the understanding of the phototrophic communities in Arctic soil ecosystems and its relation to environmental factors. In comparison with future observations in the Open Top Chambers, it could be useful to predict climate change impact in the Arctic.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in GenBank with accession numbers MK929233-MK929255 and in Sequence Read Archive (SRA) with accession number PRJNA561039.

AUTHOR CONTRIBUTIONS

EP and JE designed the study and collected the samples. EP performed laboratory work and data analyses. KL provided meteorological data and participated in the meteorological data analysis. AW contributed to the molecular part of the study. EP wrote the manuscript. All authors performed the revision.

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SUPPLEMENTARY MATERIAL

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Whole Genome Shotgun Sequencing Detects Greater Lichen Fungal Diversity Than Amplicon-Based Methods in Environmental Samples

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In this study we demonstrate the utility of whole genome shotgun (WGS) metagenomics in study organisms with small genomes to improve upon amplicon-based estimates of biodiversity and microbial diversity in environmental samples for the purpose of understanding ecological and evolutionary processes. We generated a database of full-length and near-full-length ribosomal DNA sequence complexes from 273 lichenized fungal species and used this database to facilitate fungal species identification in the southern Appalachian Mountains using low coverage WGS at higher resolution and without the biases of amplicon-based approaches. Using this new database and methods herein developed, we detected between 2.8 and 11 times as many species from lichen fungal propagules by aligning reads from WGS-sequenced environmental samples compared to a traditional amplicon-based approach. We then conducted complete taxonomic diversity inventories of the lichens in each one-hectare plot to assess overlap between standing taxonomic diversity and diversity detected based on propagules present in environmental samples (i.e., the "potential" of diversity). From the environmental samples, we detected 94 species not observed in organism-level sampling in these ecosystems with high confidence using both WGS and amplicon-based methods. This study highlights the utility of WGS sequence-based approaches in detecting hidden species diversity and demonstrates that amplicon-based methods likely miss important components of fungal diversity. We suggest that the adoption of this method will not only improve understanding of biotic constraints on the distributions of biodiversity but will also help to inform important environmental policy.

Keywords: metagenomics, ribosomal RNA, sequence database, biological soil crusts, lichen, potential of diversity, taxonomic diversity

INTRODUCTION

Microbial diversity present in the environment is recognized increasingly for its important and varied roles in the health of ecosystems (Chen et al., 2018; Nottingham et al., 2018; Pike et al., 2018), particularly in the face of a changing climate (Cavicchioli et al., 2019). Unsurprisingly, a great deal of focus has been on quantifying biodiversity—the number, identity, and functions of species (Gotelli and Colwell, 2001; Faith, 2002; Barlow et al., 2007). It has also helped researchers more fully document the ranges of rare or endangered species through environmental DNA detection strategies (Olson et al., 2012; Thomsen et al., 2012; Spear et al., 2015). Further expanding the impacts of this relatively new field, microbial metagenomics has proven exceptionally useful toward informing remediation strategies of disturbed habitats such as ecologically sensitive biological soil crusts (BSC; Bowker, 2007; Steven et al., 2012). Failure to fully understand the microbial (biotic) community can thus dramatically limit understanding of what structures ecological interactions, species distributions, and environmental sustainability, all of which can, in turn, negatively impact informed conservation decision making (Guisan et al., 2013).

Increased accessibility and affordability of high throughput sequencing, has facilitated broad scale exploration of microbial community structure (Logares et al., 2012, 2014; Chen et al., 2017; Zhang et al., 2018). At present, the majority of broad-scale biotic diversity assessments employ primarily culture-independent, amplicon-based sequencing (Petrosino et al., 2009; Mande et al., 2012; Uyaguari-Diaz et al., 2016). This method relies on sufficiently variable, universally present regions of the genome, or "barcoding loci" (Hebert and Gregory, 2005; Kress and Erickson, 2008). Such loci must, first and foremost, be unique enough to yield distinctions between species present in a sample (Kolbert et al., 2004). A proliferation of bioinformatic pipelines developed for barcode sequencing has resulted in widespread capacity to analyze microbial diversity and community structure present in a variety of different environments, ranging from soil to the human body (e.g., QIIME; Caporaso et al., 2010; Kuczynski et al., 2012; Navas-Molina et al., 2013). These pipelines rely primarily on the 16S ribosomal DNA (rDNA) gene as a target for PCR amplification and subsequent sequencing to distinguish species (Winker and Woese, 1991; Kolbert et al., 2004; Petrosino et al., 2009). However, shortcomings of such amplicon-based approaches include moderate to extreme amplification bias (Acinas et al., 2005; Wang and Qian, 2009), thus effectively investigating only a fraction of total standing diversity.

Fungi decompose organic litter (Chapin et al., 2002; Osono, 2007), produce secondary compounds of tremendous importance to humans such as antibiotics (Keller et al., 2005), are used extensively in food production (e.g., bread, wine, beer; Campbell-Platt and Cook, 1989), and are common agricultural pests (e.g., *Sclerotinia sclerotiorum*; Amselem et al., 2011). Despite their immense ecological (Bever et al., 2001; Pitt and Hocking, 2009; Van Der Heijden and Horton, 2009) and economic importance (Sharma, 1989), fungal metagenomics has, on the whole, received less attention relative to bacterial metagenomics. For example, only 360 NCBI Bioprojects were archived for fungi using amplicon-based molecular barcodes compared to 5,121 Bioprojects available for studies of microbes using amplicon-based molecular barcodes (search conducted on June 21, 2019, in NCBI Bioproject Archive).

Lichens are a species-rich and evolutionarily heterogeneous assemblage of fungi that form obligate symbioses with a minimum of one primary photosynthetic partner, often in addition to other endolichenic fungi, algae, and bacteria (Ahmadjian and Jacobs, 1981; Seaward, 1997; Brodo et al., 2001; Papazi et al., 2015). Lichens are highly successful and ecologically important, as is evidenced by their abundance and diversity in terrestrial ecosystems around the world (Hawksworth, 1991). Along with bryophytes, cyanobacteria, and non-lichenized fungi, lichens are a crucial component of biological soil crust communities and function prominently in ecological restoration processes (Belnap, 2001; Belnap and Lange, 2001; Thompson et al., 2006; Bowker, 2007). In addition to their pivotal ecological contributions to such communities, lichenized fungi have relatively small metagenome sizes (Armaleo and May, 2009; Tripp et al., 2017), making them ideal targets for cost-effective genomics projects (Allen et al., 2018; Brigham et al., 2018; Funk et al., 2018; Pogoda et al., 2018, 2019). Given the above, lichens serve as an excellent system in which to explore the factors that constrain the establishment and development of obligate symbioses in nature, including those prevalent among soil crust communities. Such factors span the dynamics of propagule dispersal, the distribution and establishment of individual symbionts in the environment, and biotic interactions between extant symbionts in a given environment. To date, however, few studies have explored such avenues of research, but these have relied entirely on amplicon-based sequencing methods (Banchi et al., 2018; Eaton et al., 2018; but see, e.g., Tripp et al., 2017; Pizarro et al., 2019).

This shortage of fungal genomic resources broadly, and lichen genomic resources more specifically, makes it challenging to investigate key questions about lichen ecology, evolution, genetics, and physiology. Moreover, existing studies that have investigated fungal metagenomic communities, like bacteria, primarily relied on amplicon-based approaches. Given known complications arising from amplification bias (Acinas et al., 2005; Wang and Qian, 2009), one potential solution is to forego amplification of barcoding loci and instead utilize data from whole-genome shotgun sequencing (WGS). This method avoids classical PCR amplification biases but has been little employed to date, likely as a function of one to several challenges. In addition to increased costs of WGS relative to amplicon-based methods, the lack of developed, publicly available reference databases (e.g., complete or nearly complete rDNA complexes) as well as a paucity of bioinformatics pipelines have limited the utility of WGS as a primary tool with which to approach fungal and other microbial metagenomic research.

In this study, we construct and then employ a new rDNA database spanning 273 species of lichenized fungi, built from a metagenomic survey of lichens in the southern Appalachian Mountain biodiversity hotspot, which is characterized by stark

abiotic gradients and is home to over a thousand species of lichens (Dey, 1978; Brodo et al., 2001; Hodkinson, 2010; Lendemer et al., 2013; Tripp and Lendemer, 2019a,b; Tripp et al., in press). Coupled with development of a new bioinformatic pipeline, we identify lichen fungal symbionts present in WGS metagenomic environmental samples and then compare the efficacy of our approach against traditional ITS1-based amplicon sequencing of the same samples. We place our results in the broader framework of intensive biodiversity inventories of lichens at the same plots from which environmental samples were taken. Drawing on resulting data, we demonstrate that detection of symbionts using amplicon-free methods, here WGS, detects more species than amplicon-based methods. We introduce a new biodiversity metric, "Potential of Diversity" (hereafter PoD), which refers to the potential for species to occur at a given site in a given study area, regardless of whether the species is actually present at this site as determined by traditional taxonomic inventory. In the present study, PoD specifically refers to the ratio of lichen fungal symbionts detected on bare surfaces in the environment that would serve as biotic partners in subsequent lichen symbiosis (i.e., the potential lichens that could occur in a study plot based on presence of the required fungal symbiont). In this context, PoD is a useful metric in which to fully consider biotic constraints that limit establishment of obligate symbiotic organisms. We compare this metric to the number of lichens detected as established symbioses (i.e., the lichens that visibly occur at a plot, henceforth "TD" for taxonomic diversity).

MATERIALS AND METHODS

Study Area, Field Plots and Field Sampling

This study was carried out in two one-hectare plots located in Great Smoky Mountains National Park, in the southern Appalachian Mountain Biodiversity Hotspot of eastern North America (**Figure 1**). The plots were selected to span the two extremes of a stark elevational (one high, one low) and ecological (bottomland hardwood vs. cloud-laden forest dominated by conifers) gradient in the region, so as to maximize the difference in extant lichen communities (i.e., minimize overlap) as well as potential environmental symbiont pools between the plots. The high-elevation (2,014 m) plot was located on the summit of Clingman's Dome in spruce-fir forest along the border of Swain County, North Carolina and Sevier County, Tennessee. The low-elevation (670 m) plot was located at White Oak Branch in mixed-hardwood forest above the north shore of Fontana Lake, in Swain County, North Carolina.

Prior to environmental sampling, data pertaining to several ecological variables (e.g., tree DBH, woody plant inventory, habitat quality assessment, following Tripp et al., 2019) were recorded in order to delimit the plant communities and to ensure maximum difference in lichen communities (see above). Both plots were delimited to be uniform in vegetation type within a plot (i.e., not spanning more than one ecotone). In each plot, a full inventory of lichen species was conducted [carried out by JL, vouchers deposited in the herbaria of the New York Botanical Garden [NY] and University of Colorado, Boulder [COLO]]; methods following (Tripp et al., 2019). In each plot, 16 environmental samples were obtained by swabbing the surfaces of eight rocks and eight trees (yielding a total of 32 environmental samples) for 30 seconds with a sterile toothbrush (size, aspect, and identification were recorded for each rock and tree type; see below for additional details). In order to avoid sampling surfaces with artificially inflated propagule counts, such as spore deposits along river beds and bogs, we chose only vertically-oriented, bare surfaces of rocks and trees

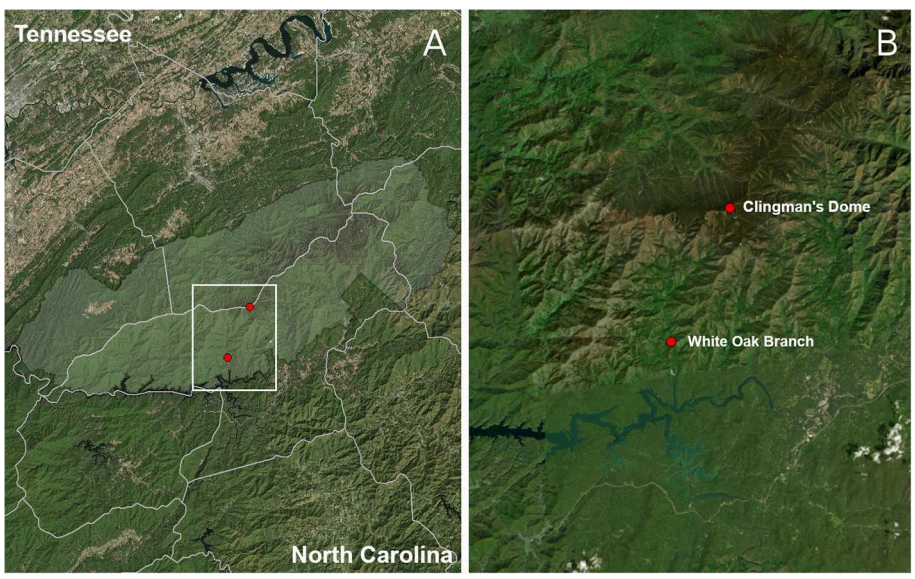

FIGURE 1 | Plot map. Area shaded in light green **(A)** corresponds to Great Smoky Mountains National Park. White Oak Branch is the low-elevation sampling plot, at 670 m elevation, and Clingman's Dome, the highest peak in Tennessee, is at an elevation of 2,014 m **(B)**.

(i.e., free of any visible growth besides the bark of the tree, when applicable).

Thallus Collection

To facilitate direct comparison of amplicon-based sequencing to WGS-based sequencing of the 32 environmental samples, we first obtained lichen voucher specimens for species present throughout the Southern Appalachian Mountains (**Table S1**) as part of a system-wide investigation of drivers of lichen biodiversity and distributions in the region, including potential biotic constraints (e.g., presence of symbionts) such as those herein investigated. These samples were collected in order to build a new genomic reference database for lichens of the region (see below). Samples were collected and identified by JCL and EAT between December 2016 and January 2018. All lichen voucher specimens are deposited at NY and COLO (**Table S1**). Efforts were made to sample only single thalli for both macro- and microlichens. For macrolichens, ca. 1 × 1 cm of thallus was removed, targeting the margins and lobes. For microlichens, thallus was scraped from rock or tree substrates using a sterile razor blade. Samples were air dried in a laminar flow hood for 24 h then frozen at −20°C until transport to the University of Colorado for DNA extraction and subsequent sequencing.

Metagenomics Sampling Scheme

To quantify the number and identification of lichenized fungi present in the 32 environmental samples as well as assess differences between amplicon- vs. WGS-based sequencing approaches, we collected samples from eight rock plus eight tree surfaces within each hectare ($n = 16$ per plot). Substrates were randomly swabbed among those available, and only bare surfaces lacking visible bryophyte or lichen thalli were chosen. Standardized stencils of 10 × 10 cm were placed against the substrate and an individually packaged, sterile toothbrush was used to swab the surface for 30 s. The toothbrush containing the sample was then sealed in a sterile plastic bag. To process the samples, the bag was opened, the bristles were cut from the brush using a sterile blade, and a hole was cut into the corner of the bag using sterile scissors. The opened corner of the bag was placed into a sterile 1.5 mL microcentrifuge tube and the bristles were directly transferred into the tube. These samples were stored at −20°C until transport to the University of Colorado for subsequent extraction and sequencing.

DNA Extraction and Whole Genome Shotgun Sequencing

For both the 32 environmental samples as well as lichen species vouchered in order to build a new reference genomic database, dried samples were pulverized using tungsten carbide bearings in a Qiagen 96-well plate shaker. Genomic DNA (gDNA) was extracted from lichen thallus samples and toothbrush bristles using a Qiagen DNeasy 96 plant kit. Individual samples were transferred from 1.5 mL microcentrifuge tubes into 96 well plates used in the Qiagen kit. The manufacturer's protocol was modified to include a 10 min 65°C incubation step for ground material in lysis buffer as well as a 100% ethanol wash before final drying of the membrane prior to elution. Preliminary study found that these modifications improved lichen gDNA concentration and purity (Pogoda et al., 2018). Extracted samples were stored at −20°C prior to subsequent library preparation.

Whole genome shotgun sequencing was conducted on a total of 494 lichen thallus libraries [these collected throughout the southern Appalachian study area (**Figure S1**)] and 32 environmental samples on the Illumina NextSeq®. Each of the gDNA samples was prepared using the Nextera® XT DNA library prep kit, which is optimized for 1 ng of total input DNA. Each sample was uniquely tagged using the dual index adapters, Nextera® i5 and i7. Libraries prepared for sequencing on the NextSeq® utilized Illumina PhiX v.3 as a control and samples that passed QC were processed for paired-end 151 base pair reads on an Illumina NextSeq® sequencer at the University of Colorado's BioFrontiers Institute (Boulder, Colorado).

ITS1 Sequencing

To compare results of WGS-based sequencing to amplicon-based sequencing, amplification by PCR was performed on the 32 environmental samples using the ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA) (Gardes and Bruns, 1993) and ITS2 (5'-GCTGCGTTCTTCATCGATGC) (White et al., 1990) primers. Libraries were prepared for sequencing on the MiSeq® utilized Illumina PhiX v.3 as a control. Samples that passed QC were then processed for single end 251 base pair reads on the Illumina MiSeq® sequencer at the University of Texas' Genomics Sequencing and Analysis Facility (Austin, Texas).

Genome Assembly and Reference Genomic Database

Libraries were filtered with Trimmomatic-0.36 to trim adapters from reads, and with parameters "LEADING:3 TRAILING:3 MINLEN:100" (Bolger et al., 2014). Filtered reads were then assembled using SPAdes 3.9.0 with parameters "–careful -k 21,33,65,81" (Bankevich et al., 2012). This study utilized the whole nuclear ribosomal DNA (rDNA) complexes that were obtained from the *de novo* genome assembly of lichens that were collected as part of a broader study of lichen diversity in the southern Appalachian study region (Keepers et al., unpub. data). The rDNA complex is easily assembled due to its high copy number in the nuclear genome, and is long (i.e., >5,000 bp, in comparison to amplicon-based sequencing of ITS1, which is <500 bp), providing a larger target onto which sequenced reads may map. Moreover, the high-copy nature of the locus provides many opportunities per propagule to be counted in the downstream analyses. The rDNA complexes for each sample were identified by conducting a BLAST search of the rDNA complex of the trebouxioid algal photobiont from *Cladonia uncialis* against each of the assemblies. The algal rDNA was used in the search rather than a mycobiont sequence to avoid bias in BLAST hit length due to phylogenetic similarity. To identify complete or mostly complete rDNA complexes from the BLAST

tables, contigs were required to have two or greater distinct hits and the span of these hits were required to be >1,000 bp in length. Sequences were parsed from the assemblies based on the nucleotide positions of the BLAST hits and oriented with the 18S in the 5' direction.

Phylogenetic Methods

Each putative rDNA contig parsed from the assemblies was BLAST searched against the NCBI non-redundant database. Any sequences whose best BLAST results mapped to non-lichenized fungi or to non-fungal species were excluded. To further vet the identities of the contigs in the database, sequences were aligned using MUSCLE aligner v3.8.31 (Edgar, 2004) for downstream phylogenetic analysis. The resulting preliminary alignment was trimmed to contain only highly homologous regions, then further trimmed to only sites for which >90% of taxa in the dataset contained sequence data. A best estimate phylogenetic tree was inferred under a model of GTR+I+Γ using MrBayes (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) with 10,000,000 MCMC generations and gaps treated as missing data. The first 25% trees were treated as burn-in and excluded from further analyses; eight runs were implemented and 32 chains were utilized. All other parameters were left at default values and the average standard deviation of split frequencies had converged to less than the recommended 0.01 by the end of the analysis. The trees from the posterior distribution were used to generate a majority consensus tree, upon which we mapped posterior probabilities.

This preliminary consensus tree was visualized using FigTree v.1.4.3, upon which we determined that 34 samples were likely misplaced based on disagreement with established large-scale phylogenic reconstructions of lichenized fungi (e.g., Miadlikowska et al., 2014). As such, these 34 samples were excluded from the alignment, which was then further pruned of duplicated taxon sequences such that the final matrix contained only one representative per species (and in each case, the longest rDNA contig was retained). The resulting final alignment included a total of 273 sequences and, after visual inspection following the same phylogenetic methods described above, was used as the reference database for subsequent queries of sequences obtained from sequencing of the environmental samples.

Read Mapping

The 251 bp amplicon reads were truncated to 151 bp to ensure comparability of amplicon-based sequences to WGS sequences. To exclude PCR primer sequence regions, amplicon reads were truncated to include only positions 50–200, representing the highly variable ITS1 sequence. Sequences from both the WGS and amplicon-based approaches were then aligned to the newly constructed reference rDNA database to generate short-read alignment maps. To ensure that reads uniquely mapped to a single locus in the database, several filtering steps were employed. First, only reads that mapped with a CIGAR score between 145 and 151 matches, corresponding to a mapping identity of between 96 and 100%, were retained. Second, only read-pairs for which both the left and right read mapped to the same contig were kept, and then only read-pairs for which both reads mapped with a SAM mapping quality of 31 or greater (out of a maximum of 60) were retained. Due to the highly conserved nature of portions of the coding regions of the ribosomal DNA complex, many reads belonging to species for which multiple congeners were represented in the database mapped almost equally well to multiple species. In these instances, the mapping score was bolstered by the estimated read separation. Thus, a higher SAM CIGAR score of 150 or 151 was required to retain reads that also mapped well to a member of the same genus.

Species Accumulation Curves

To facilitate comparison of species diversity and accumulation as assessed from environmental samples, species richness rarefaction curves for each of the 32 samples falling under four sampling regimes (i.e., eight each from high elevation rock, high elevation tree, low elevation rock, low elevation tree) were generated using the Diversity Stats calculator in EstimateS 9.1.0 (Colwell, 2013). Rarefaction curves were bootstrapped by randomizing the sample order 100 times.

Comparison of Environmental Sampling Methods and Expert-Based Inventory

To assess the congruence between the two metagenomic methods (i.e., WGS vs. amplicon) of detecting symbionts in environmental samples (potential of diversity, or "PoD") as well as congruence of both methods to expert-based inventory (Coddington et al., 1991; Sørensen et al., 2012) of species found growing in each plot (taxonomic diversity, or "TD"), we pooled the taxa detected in all samples collected in each of the four sampling regimes (high rock, high tree, low rock, low tree) to produce a single list of species detected through a given method) and calculated Jaccard indices in each inventory. These were derived from a presence/absence matrix that consisted of the species present in the rDNA reference database that were found at each of the two elevation extremes via (1) the expert-based lichen biodiversity inventory, (2) the lichenized fungi detected in environmental samples using amplicon-based sequencing, and (3) the lichenized fungi detected in environmental samples using WGS sequencing. Similarity between the lichens detected with the different methods is reported below as $J_{Treatment,Treatment}$ (e.g., $J_{WGS,Vouchered}$ is similarity of lichenized fungi detected by WGS of environmental samples compared to that detected by expert-based inventory).

RESULTS

Ribosomal DNA Database

The final reference genomic database of lichen rDNA contained complete or nearly complete ribosomal DNA complexes (NTS, ETS, 18S, ITS1, 5.8S, ITS2, 26/28S) for 273 unique species of lichenized fungi within Pezizomycotina, representing 25 orders

and 57 families. The database spanned 1,770,139 bp of sequence, with an average contig length of 6,484 bp.

Detection of Lichenized Fungi Using Expert, WGS, and Amplicon-Based Inventory

Taxonomic diversity (TD: the number of vouchered species) of lichens found growing in the study plots totaled 57 species in the high-elevation plot and 83 species in the low-elevation plot, yielding a total of 136 species in both ($n = 4$ species occurred in both). Of these 136 species, 78 (∼60%) were represented in the rDNA database generated in this study and thus could potentially be matched to sequences from the environmental samples. Note that the additional species in the database were vouchered from other plots in the study area and were used to ascertain whether this method detected species that were not vouchered in the expert-based field inventory.

Mapping of paired-end WGS reads to the rDNA reference database resulted in the detection of a total of 94 lichenized fungi present in both plots: 43 species from the high-elevation plot and 71 from the low-elevation plot (**Table 1**). Conversely, using the traditional amplicon-based approach of mapping ITS1 reads to the rDNA reference database resulted in the detection of a total of 34 lichenized fungi present in both plots: 21 species from the high-elevation plot and 18 species from the low-elevation plot (**Table 1**). Species detection accumulated consistently faster by sample using WGS in all four sampling regimes (**Figure 2**).Of the 57 species vouchered during the inventory of the high-elevation plot, 13 were detected using both sequencing methods although only seven of these species represented the same species between the two plots (**Figure 3**; **Table 1**). Conversely, of the 83 species that were vouchered during the inventory of the low-elevation plot, 17 were detected using either sequencing method, although only 4 were detected by both (**Figure 3**; **Table 1**).

The similarity between the community of lichens detected growing at the plots via expert-based inventory vs. the pools of lichen fungal symbionts species detected in environmental samples using WGS or amplicon sequencing are reported as Jaccard indices in **Table 2**. The similarity values for the assemblages of lichenized fungi detected in the environment at the high-elevation plot compared to those vouchered in the expert-based inventory ($J_{WGS,Vouchered} = 0.148$, $J_{AMP,Vouchered} = 0.200$) were roughly twice as high as their respective indices at the low-elevation plot ($J_{WGS,Vouchered} = 0.108$, $J_{AMP,Vouchered} = 0.063$). This result conveys a greater congruence between the PoD inventory based on WGS sequencing to the TD inventory, than the PoD inventory based on amplicon sequencing to the TD inventory.

DISCUSSION

Our results demonstrate that, in comparison to amplicon sequencing, the WGS approach detects a greater number of species that exist within an environment as propagules. When analyzed in tandem with data from expert-based taxonomic

TABLE 1 | Inventory of species of lichenized fungi collected at the high-elevation Clingman's Dome and low-elevation White Oak Branch plots.

	High elevation (Clingman's Dome)			Low elevation (White Oak Branch)		
	Vouchered	**WGS**	**Amplicon**	**Vouchered**	**WGS**	**Amplicon**
Acanthothecis sp.		■			■	
Anaptychia palmulata				■		
Anisomeridium sp.					■	
Anzia colpodes		■				
Arthonia anglica				■	■	
Arthonia ruana					■	
Arthonia rubella					■	
Aspicilia laevata					■	
Bacidia heterochroa				■		
Bacidia schweinitzii				■		
Bacidia sorediata					■	■
Bacidia sp.					■	
Baeomyces rufus	■					
Biatora appalachensis	■	■	■		■	
Biatora pontica			■		■	■
Biatora printzenii				■		
Botryolepraria lesdainii					■	
Brigantaea leucoxantha					■	
Bryoria bicolor	■		■			
Bryoria furcellata	■					
Bryoria nadvornikiana	■					
Bryoria tenuis	■					
Buellia stillingiana				■		
Buellia vernicoma				■		
Bulbothrix scortella		■				
Caloplaca camptidia		■		■	■	
Cetrelia cetrarioides	■					
Cetrelia chicitae	■				■	
Cladonia arbuscula		■				
Cladonia coniocraea				■		
Cladonia didyma				■		
Cladonia macilenta	■	■				
Cladonia mateocyathea					■	
Cladonia ochrochlora	■		■	■		
Cladonia parasitica	■					
Cladonia peziziformis				■		
Cladonia polycarpoides					■	
Cladonia pyxidata			■			■
Cladonia robbinsii		■			■	
Cladonia squamosa	■	■	■			
Cladonia strepsilis			■			
Cladonia subtenuis		■				
Coccocarpia palmicola				■		
Collema subflaccidum				■		

(Continued)

TABLE 1 | Continued

	High elevation (Clingman's Dome)			Low elevation (White Oak Branch)		
	Vouchered	WGS	Amplicon	Vouchered	WGS	Amplicon
Dendriscocaulon intricatulum					■	
Everniastrum catawbiense	■	■	■			
Flavoparmelia baltimorensis					■	
Flavoparmelia caperata	■					
Fuscopannaria leucosticta				■		
Graphis scripta				■	■	
Gyalideopsis ozarkensis					■	
Gyalideopsis piceicola	■					
Heterodermia casarettiana				■		■
Heterodermia hypoleuca					■	
Heterodermia obscurata				■		
Heterodermia squamulosa				■		
Hypogymnia incurvoides	■	■				
Hypogymnia krogiae	■	■	■			
Hypogymnia vittata	■	■				
Hypotrachyna afrorevoluta	■					
Hypotrachyna croceopustulata	■					
Hypotrachyna gondylophora	■					
Hypotrachyna horrescens				■		
Hypotrachyna imbricatula	■					
Hypotrachyna livida				■		
Hypotrachyna minarum		■			■	
Hypotrachyna oostingii	■					
Hypotrachyna prolongata	■					
Hypotrachyna showmanii				■		
Hypotrachyna thysanota	■					
Hypotrachyna virginica	■					
Icmadophila ericetorum	■					
Imshaugia aleurites	■		■			
Ionaspis alba					■	
Lecanora appalachensis		■			■	■
Lecanora cinereofusca	■				■	
Lecanora hybocarpa					■	

(Continued)

TABLE 1 | Continued

	High elevation (Clingman's Dome)			Low elevation (White Oak Branch)		
	Vouchered	WGS	Amplicon	Vouchered	WGS	Amplicon
Lecanora imshaugii				■		
Lecanora masana	■		■		■	
Lecanora nothocaesiella				■		
Lecanora rugosella	■	■	■		■	
Lecanora saxigena					■	
Lecanora symmicta	■					
Lecanora thysanophora				■		
Lecidea berengeriana				■		
Lecidea roseotincta	■		■			
Lecidea tessellata		■				
Lecidella sp.		■				
Lepra pustulata					■	
Lepraria oxybapha	■	■	■		■	■
Lepraria sp.					■	
Lepraria vouauxii				■		
Lepraria xanthonica				■		
Lepra trachythallina					■	
Leptogium corticola				■		
Leptogium cyanescens				■		
Leptogium dactylinum				■		
Leptogium hirsutum		■			■	
Lobaria pulmonaria				■	■	
Lopadium disciforme	■					
Loxospora elatina		■	■			
Loxospora ochrophaea	■					
Megalospora porphyritis				■	■	■
Melanohalea halei	■				■	
Menegazzia subsimilis				■		
Micarea neostipitata		■			■	■
Micarea peliocarpa				■		
Multiclavula mucida				■		
Mycoblastus caesius	■					
Mycoblastus sanguinarioides	■					
Mycocalicium subtile		■			■	
Myelochroa aurulenta				■		
Myelochroa galbina				■		
Nephroma helveticum				■		
Ochrolechia trochophora				■	■	
Opegrapha viridis				■		
Opegrapha vulgata		■			■	
Pannaria tavaresii		■			■	
Parmelia saxatilis	■					

(Continued)

TABLE 1 | Continued

	High elevation (Clingman's Dome)			Low elevation (White Oak Branch)		
	Vouchered	WGS	Amplicon	Vouchered	WGS	Amplicon
Parmelia squarrosa				■		
Parmotrema arnoldii	■				■	
Parmotrema cetratum			■		■	■
Parmotrema diffractaicum				■		
Parmotrema gardneri				■		
Parmotrema hypotropum				■	■	
Parmotrema margaritatum		■				
Parmotrema perforatum				■		
Parmotrema perlatum						■
Parmotrema reticulatum					■	
Parmotrema simulans					■	■
Parmotrema subisidiosum				■		
Parmotrema submarginale				■		
Pertusaria andersoniae					■	
Pertusaria macounii				■		
Pertusaria obruta		■		■		
Pertusaria ostiolata					■	
Pertusaria paratuberculifera				■		
Pertusaria plittiana					■	
Pertusaria rubefacta		■			■	
Pertusaria subpertusa				■		
Pertusaria texana				■		
Phaeophyscia adiastola					■	
Phlyctis boliviensis				■	■	■
Phyllopsora corallina				■	■	
Physcia stellaris				■		
Placynthiella icmalea	■					
Platismatia glauca	■	■			■	
Platismatia tuckermanii	■	■				
Porina scabrida				■		
Porpidia albocaerulescens	■	■	■	■	■	■
Porpidia crustatula			■			
Pseudevernia cladonia	■		■			
Pseudevernia consocians	■					
Pseudocyphellaria aurata				■	■	
Pseudosagedia isidiata		■		■	■	

(Continued)

TABLE 1 | Continued

	High elevation (Clingman's Dome)			Low elevation (White Oak Branch)		
	Vouchered	WGS	Amplicon	Vouchered	WGS	Amplicon
Pseudosagedia rhaphidosperma				■		
Punctelia rudecta				■		
Pyrenula pseudobufonia					■	
Pyrrhospora varians				■	■	
Pyxine sorediata				■		
Ramalina culbersoniorum				■		
Rhizocarpon geographicum					■	
Rhizocarpon infernulum				■		
Rinodina ascociscana		■		■		
Rinodina buckii				■		
Rinodina subminuta				■		
Ropalospora chlorantha	■				■	■
Ropalospora viridis				■		
Sarea resinae		■			■	
Scoliciosporum umbrinum		■				
Stereocaulon dactylophyllum		■				
Sticta beauvoisii				■		
Sticta sp.						■
Strigula stigmatella				■		
Thelotrema subtile					■	
Trapelia coarctata		■		■	■	
Trapelia placodioides			■			
Trapeliopsis flexuosa	■					
Trapeliopsis sp.		■				
Trapeliopsis viridescens	■	■			■	
Trypethelium virens				■		
Tuckermanopsis ciliaris		■				
Umbilicaria mammulata					■	
Unknown SSC (FEN 213)		■				
Usnea cornuta	■					
Usnea dasopoga	■					
Usnea merrillii	■					
Usnea mutabilis					■	
Usnea pensylvanica				■		
Usnea strigosa				■		■
Usnea subgracilis				■	■	■
Usnea subscabrosa					■	■
Usnocetraria oakesiana	■			■		
Vainionora americana				■		
Varicellaria velata				■		
Variolaria amara				■		

(Continued)

TABLE 1 | Continued

	High elevation (Clingman's Dome)			Low elevation (White Oak Branch)		
	Vouchered	WGS	Amplicon	Vouchered	WGS	Amplicon
Variolaria pustulata				■		
Variolaria trachythallina	■					
Variolaria waghornei				■		
Xylographa truncigena		■	■			
Xylographa vitiligo	■					

inventories of the same locations, there is an added capability to compare the number and community composition of extant, fully formed symbioses that occur in nature, to those that could potentially occur based on the pool of symbionts in the environment. In turn, this PoD-to-TD comparison helps unravel biotic constraints on the distributions of biodiversity. The estimation of species counts and taxonomic diversity using WGS metagenome sequencing has been conceptually validated in planktonic microbial communities (Poretsky et al., 2014). However, to our knowledge, the application of this method to macro-eukaryotes is only just emerging (Donovan et al., 2018). This study provides a substantial increase in the number of rDNA sequences from lichenized fungi now made available for future research. At the time of writing (April 2019), there were 779 complete (>3,000 bp) ribosomal DNA sequences within the Pezizomycotina (if "18S," "complete," and either "26S" or "28S" are required in the search) publicly available on GenBank. Accounting for the 49 complete lichenized fungal ribosomal DNA sequences that had already been submitted from the database curated here, our additional 224 new sequences represent a 29% increase in genomic resources for this locus for lichens.

Our workflow presented herein adds to the growing toolset of molecular contributions to biodiversity science. We have demonstrated the utility of using WGS metagenomic libraries to estimate the pool of available symbionts that are present in an environment. Given the expected ongoing decreases in sequencing cost (Schuster, 2007), we anticipate that WGS will be readily adopted in many study systems and organisms.

Understanding the Distributions of Lichens and Their Propagules

At both sampling plots (high-elevation and low-elevation), species were detected based on environmental (PoD) sampling that were not present based on TD sampling. In other words, lichen mycobionts were detected in the environmental samples but were not detected by the inventory of lichens growing at the site. There are two plausible explanations for these results. First, and likely applicable to most such instances, species detected in the environmental samples but not in the inventories were present only as propagules derived from other locations. It has been shown that the gametes of sexually reproducing lichens can disperse several kilometers (Ronnås et al., 2017), and even clonal propagules are capable of dispersing over a kilometer (Gjerde et al., 2015; Eaton et al., 2018), although these studies suggest that such long-distance dispersal events are rare relative to the total reproductive propagule output of any given individual lichen thallus. It is thus not unexpected that the bank of lichen propagules on a given surface could include representatives of a more diverse pool of lichen species, these derived from a broader geographic neighborhood, regardless of the suitability of the substrate for colonization by those species. Indeed, similar patterns of have been recovered from amplicon sequencing of lichen propagules from indoor dust samples in the United States (Tripp et al., 2016).

The presence of a lichenized fungal propagule alone represents only one element of the total biotic community needed for a lichen species to grow into a mature individual, with the presence or absence of other algal, bacterial, and or other fungal species potentially representing constraints to development of a given lichen thallus (see Tripp et al., 2019). Other constraints include abiotic factors such as elevational or precipitation regimes selected for by different lichen species. One example of this phenomenon in our dataset is *Bulbothrix scortella*, which was detected only in WGS sequencing of the environmental samples from the high-elevation plot. *Bulbothrix scortella* is a subtropical species that occurs only at low-elevations in the southern Appalachian Mountains (Hale, 1976; Lendemer et al., 2013). As such, the detection of the species with only WGS sequencing likely reflects the dispersal of a lichenized fungal propagule to the high-elevation plot (from nearby low-elevation habitats), where it is unlikely to become established and undergo further development owing to abiotic and/or biotic constraints.

A second and less likely explanation for our results is failure to detect the presence of a lichen using expert-based TD inventories. Lichens are widely recognized as microhabitat specialists with many species present as small or spatially restricted populations in a given geographic area (Peck et al., 2004; Belinchón et al., 2015; Boch et al., 2016; Dymytrova et al., 2016). Examples from our own work have demonstrated that in some instances, inventories of the same area by more than one collector will yield < 50% overlap in the cohorts of documented species (Lendemer et al., 2016). In the present study, *Loxospora elatina* is a species known to have a distribution restricted to temperate boreal forests (i.e., high elevation habitats in the southern Appalachian Mountains) in North America and Europe (Tønsberg, 1992; Lendemer, 2013). This species was detected by both sequencing methods in the high-elevation plot, but not found in the TD inventory. Thus, it is possible that in a limited number of instances, the species detected in the environmental samples but not in TD inventories were present in low abundance and not detected. However, our expert-based inventories for the broader research project under which the current study falls (i.e., investigating drivers of diversity and distributions of lichens in the southern Appalachian Mountains) target exhaustive sampling until full vouchering of the entire pool of species diversity has been accomplished (Tripp et al., 2019).

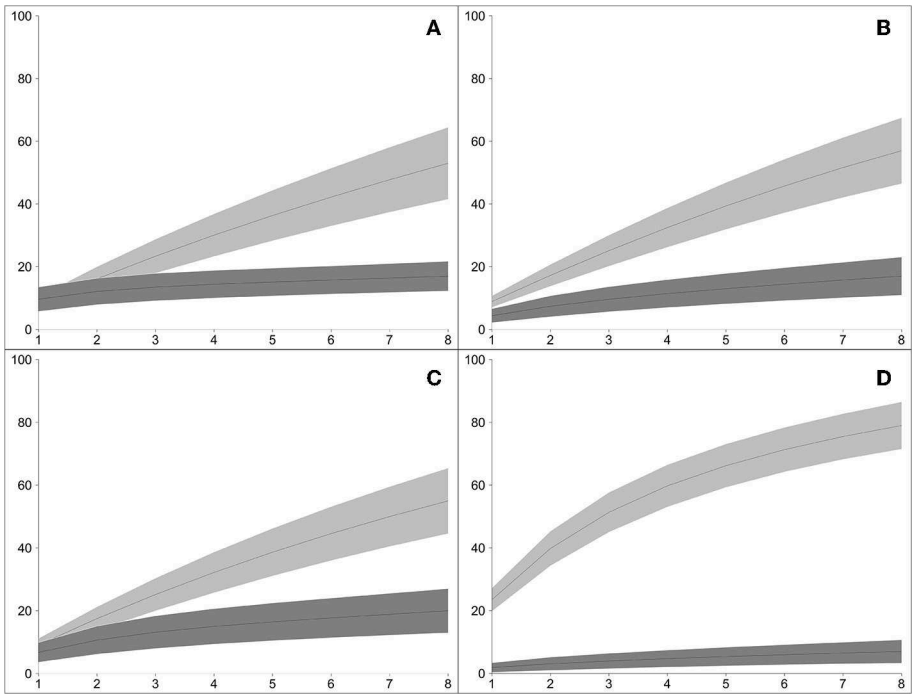

FIGURE 2 | Bootstrapped rarefaction curves of the accumulation of species of lichenized fungi, comparing WGS-based rDNA approach (light gray bands) vs. amplicon-based ITS1 approach (dark gray bands), in the four sampling regimes in two plots: **(A)** high-elevation rock samples; **(B)** low-elevation rock samples; **(C)** high-elevation tree samples; **(D)** low-elevation tree sample (x-axis: number of environmental samples; y-axis: number of species of lichenized fungi detected). In all four sampling regimes, the WGS-based approached developed in this study detected greater species diversity than the amplicon-based approach.

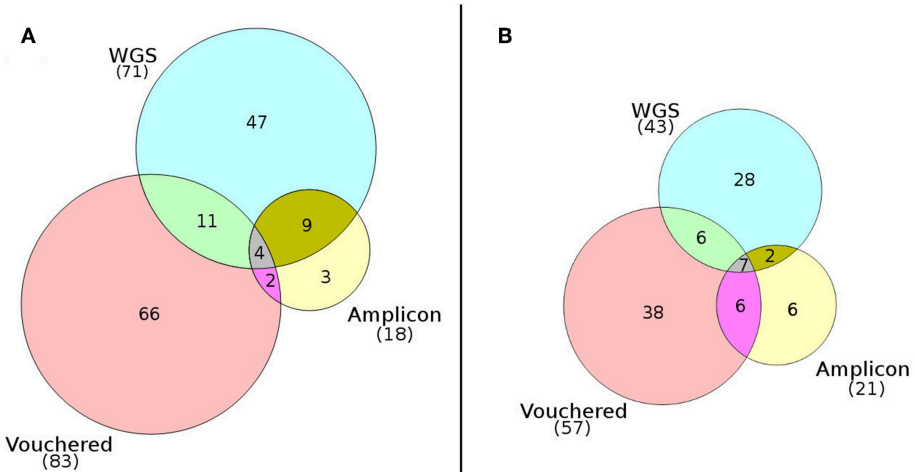

FIGURE 3 | Venn diagrams comparing the numbers of species of lichenized fungi detected at **(A)** the low-elevation White Oak Branch plot and **(B)** the high-elevation summit of Clingman's Dome. Diagrams show an overall low overlap of detected species using the WGS approach (PoD), the amplicon approach (PoD), and the expert-based inventory approach (TD).

Although replicate sampling of additional high- and low-elevation plots was not carried out in this study, we nonetheless detected clear differences in the cohorts of species between the two elevational extremes with all methods herein employed. High- and low-elevations in the southern Appalachians host different lichen communities that share in common only a small number of species (Lendemer and Tripp, 2008; Allen and Lendemer, 2016; Muscavitch and Lendemer, 2016; Lendemer

TABLE 2 | Comparison of the congruence between the species detected by either method and the vouchered species at each plot.

Jaccard indexes (High Elevation)		
	Vouchered	**Amplicon**
Amplicon	0.200	
WGS	0.148	0.161
Jaccard indexes (Low Elevation)		
	Vouchered	**Amplicon**
Amplicon	0.063	
WGS	0.108	0.171

et al., 2017; Tripp and Lendemer, 2019a,b; Tripp et al., 2019). Thus, differences between the inventories of the two plots are to be expected. Some of the differences in the cohorts of species detected through environmental sampling may reflect the overall dispersal limitation of lichen propagules that leads the propagule pool to be dominated by locally occurring species. We hypothesize that at higher elevations one would expect the detection of fewer "long distance dispersal" events such as is described for *B. scortella* above, when compared to lower elevations. The ability of a propagule to disperse is influenced in part by gravity (Ronnås et al., 2017), and thus propagules will face an uphill battle in dispersing upward through passive wind dispersal.

Benefits and Limitations of WGS vs. Amplicon Sequencing

Our analyses detected approximately four times as many species using the WGS approach compared to the amplicon-based method in the low-elevation plot, and twice as many in the high-elevation plot. The higher number of species detected by WGS affords a stricter threshold for what counts as a detected species in the SAM file (e.g., by only counting species as detected if they occur at least a certain number of times throughout the SAM file, or by requiring a higher SAM mapping quality score before counting the species as detected).

The community present in a metagenomics sample contains the DNA of species that are represented by many cells in some cases, and potentially by a single cell in other cases. Such stark differences in input quantity from different species in the sample leads to potential issues for both WGS and amplicon environmental sequencing methods. While both methods rely on low abundance DNA being extracted in sufficient quality and quantity to be detected in downstream analysis, amplicon-based metagenomics suffers from difficulties in primer design, including issues with the use of universal primers (Acinas et al., 2005; Wang and Qian, 2009). Moreover, unequal (biased) amplification is of wide concern in skewing the distribution of amplicons in the PCR product (Acinas et al., 2005; Sipos et al., 2010). Conversely, one potential issue with WGS metagenomics is that for species with low abundance in the sample, the probability of sampling that species in the prepared library will scale inversely with the proportion of the prepared library that is sequenced. For these reasons, the cohorts of species detected in the community may differ minimally to substantially between the two methods.

One complication of our approach is the presence of group I introns (DePriest and Been, 1992; DePriest, 1993; Gargas et al., 1995) in the coding sequences (18S, 5.8S, and 28S) of many lichenized fungal ribosomal complexes. These introns may be horizontally transferred between species (Hibbett, 1996; Fitzpatrick, 2012; Roy and Irimia, 2012). Thus, it is possible that a read originating from a group I intron in the rDNA complex of a species that is not present in our dataset may appear to map to the database to a species that recently received a horizontal transfer of that intron. However, this will occur very rarely, and even less-so as the database improves, for several reasons. First, our heuristics for read filtering are fairly strict, requiring read-pairs to uniquely map with high percent-identity to members of the database. Unless the intron transferred very recently, the rapid evolution of such introns (Dujon, 1989; Gargas et al., 1995; Roy and Irimia, 2012) would prevent the false detection of a species within the database. Moreover, these introns may facilitate a higher rate of species detection, due to their rapid evolution relative to the coding regions that are too conserved to uniquely identify species in many cases.

Moving forward, we aim to implement the approach presented here to measure the congruence between the observed TD of lichens in the southern Appalachians and the PoD measured in the propagule bank, as one step forward in understanding potential biotic constraints on the distributions of lichen biodiversity. The environmental covariates that predict this congruence may enable the development of cost-effective conservation measures in ecologically sensitive keystone systems such as biological soil crusts (Eldridge, 2000; Belnap, 2003; Belnap and Lange, 2013). We propose that the WGS method presented here should be added to the toolset used by molecular taxonomists in pursuit of conservation and restoration efforts, in addition to facilitating more general understanding on what structures the distributions of species. The datasets and bioinformatic resources presented here represent an important set of new tools and approaches that can be used to address a broad range of questions.

DATA AVAILABILITY STATEMENT

The database consisting of the complete (or nearly complete) ribosomal DNA complexes of 273 lichen mycobionts is available at Zenodo record #3256645. The WGS and amplicon metagenomics libraries are published on the SRA database, SUB5873533.

AUTHOR CONTRIBUTIONS

KK conducted the research, analyzed the data, and interpreted the data. KK, CP, ET, and JL wrote the paper. ET, JL, CM, and NK conceptualized the project and secured and managed funding. JL, ET, KK, KW, CA, and JH collected and vouchered samples. Processing, curation, and archiving of voucher specimens and field data was managed by AR. KK, KW, CP, and CA performed the extractions and library preps. NK helped develop the bioinformatics pipeline and edited the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00484/full#supplementary-material

Figure S1 | The southern Appalachian study area spans the states of Alabama, Georgia, Tennessee, South Carolina, North Carolina, Virginia, and West Virginia.

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The Burning of Biocrusts Facilitates the Emergence of a Bare Soil Community of Poorly-Connected Chemoheterotrophic Bacteria With Depressed Ecosystem Services

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Wildfires destabilize biocrust, requiring decades for most biological constituents to regenerate, but bacteria may recover quickly and mitigate the detrimental consequences of burnt soils. To evaluate the short-term recovery of biocrust bacteria, we tracked shifts in bacterial community form and function in Cyanobacteria/lichen-dominated (shrub interspaces) and Cyanobacteria/moss-dominated (beneath *Artemisia tridentata*) biocrusts 1 week, 2 months, and 1 year following a large-scale burn manipulations in a cold desert (Utah, USA). We found no evidence of the burned bacterial community recovering to a burgeoning biocrust. The foundational biocrust phyla, Cyanobacteria, dominated by *Microcoleus viginatus* (Microcoleaceae), disappeared from burned soils creating communities void of photosynthetic taxa. One year after the fire, the burned biocrust constituents had eroded away and the bare soils supported the formation of a convergent community of chemoheterotrophic copiotrophs regardless of location. The emergent community was dominated by a previously rare *Planococcus* species (family Planococcaceae, Firmicutes) and taxa in the Cellulomonadaceae (Actinobacteria), and Oxalobacteraceae (Betaproteobacteria). Previously burnt soils maintained similar levels of bacterial biomass, alpha diversity, and richness as unburned biocrusts, but supported diffuse, poorly-interconnected communities with 75% fewer species interactions. Nitrogen fixation declined at least 3.5-fold in the burnt soils but ammonium concentrations continued to rise through the year, suggesting that the exhaustion of organic C released from the fire, and not N, may diminish the longevity of the emergent community. Our results demonstrate that biocrust bacteria may recover rapidly after burning, albeit along a different community trajectory, as rare bacteria become dominant, species interconnectedness diminishes, and ecosystem services fail to rebound.

Keywords: biological soil crust, *Bromus tectorum*, disturbance, Great Basin Desert, rare biosphere, network co-occurrence model

INTRODUCTION

Fire may dramatically alter soil bacteria communities depending on the biome being burned (Pressler et al., 2019); fire characteristics [e.g., type (i.e., low-intensity vs. high-intensity, Xiang et al., 2014; Koster et al., 2016) and frequency (e.g., single vs. multiple, Hawkes and Flechtner, 2002; Guenon and Gros, 2013)], and depth of burned soil (Kim et al., 2004). Following the fire, burned communities begin to recover, with recovery defined as a return in biomass, community composition, and/or function to original levels prior to the disturbance. The rate of recovery, in any form, is moderated by a series of interacting factors, such as soil hydrophobicity (Faille et al., 2002; Fernelius et al., 2017), rainfall intensity and frequency (Guenon and Gros, 2013; Hinojosa et al., 2019), nutrient concentrations (Prendergast-Miller et al., 2017; Rodriguez et al., 2017), and soil erodibility (Williams et al., 2012). Within deserts, the impact of wildfires on soil bacteria is potentially immense. The surfaces of desert soils are often covered with biocrusts, which are complex mosaics of Cyanobacteria, other bacteria, green algae, lichens, mosses, and fungi. Biocrusts are autochthonally driven with photosynthate and fixed N_2 from Cyanobacteria and organic C from other photosynthetic organisms creating a nutrient-rich zone, the "cyanosphere" (Couradeau et al., 2019; Warren et al., 2019) that supports a relatively high level of bacterial biomass and diversity (Chilton et al., 2018). However, these "living skins of the desert" occupy soil surfaces in close proximity to fuels (e.g., shrubs and grass litter, woody debris) that readily burn (Hilty et al., 2003; Balch et al., 2013) and biocrust constituents themselves are often desiccated and may burn during fire. If burned, biocrusts may lose the ability to armor soils against wind and water erosion (Eldridge and Leys, 2003; Rodriguez-Caballero et al., 2015), enhance hydrologic function (Chamizo et al., 2016), and fix N for chronically N-poor desert systems (Belnap, 2002). Taken together, the loss of biocrusts to fire may detrimentally alter desert ecosystem form and function.

Wildfires may kill many biocrust constituents, but bacteria, in particular Cyanobacteria, may recover more quickly. A lichen- or moss-dominated biocrust may require decades to fully recover depending on disturbance type, intensity, and precipitation variability (Johansen et al., 1984; Belnap, 2003; Root et al., 2017). However, soil bacteria are relatively resistant to fire even among other soil biota (e.g., fungi and mesofauna, Pressler et al., 2019). For example, surviving bacteria in burnt soils may enter a state of dormancy to weather the harsh conditions induced by fire. Dormancy is extremely common bet-hedging strategy, with upwards of 90% of microbial biomass and 50% of all bacterial taxa potentially being dormant at a given time (Alvarez et al., 1998; Lennon and Jones, 2011; Wang et al., 2014). Cyanobacteria may become dormant (Rajeev et al., 2013) and survive fires by potentially employing hydrotaxis to recolonize disturbed soils (Pringault and Garcia-Pichel, 2004). Besides dormancy, bioaerosols and unburned soils immediately below the burn may serve as seed banks to aid in biocrust recovery. Bioaerosols in dust harbor an immense diversity of bacteria (Choudoir et al., 2018; Dastrup et al., 2018) and may retain a taxonomical signature of the originating soil surfaces (Boose et al., 2016; Weil et al., 2017; Dastrup et al., 2018). Further, *Microcoleus vaginatus*, the foundational Cyanobacterium in many cold desert biocrusts (Garcia-Pichel et al., 2013), is a pioneering primary producer (Belnap, 2002) that may recolonize soils from dust, provide photosynthate, and shape the heterotrophic bacterial community. Soils millimeters below burnt soils harbor some of the same taxa (Steven et al., 2013; Maier et al., 2014) and may serve as inoculum for recovering biocrusts. In the short-term, bacteria components of biocrusts have the potential to recover relatively rapidly.

The recovering Cyanobacteria-dominated biocrust may mitigate the detrimental ecosystem consequences of burnt soil surfaces. Cyanobacteria colonize the top millimeters of soils, physically weaving soil particles together with sheathed filaments and, along with other bacteria, produce exopolymeric substances that glue soil particles together (Mazor et al., 1996; Costa et al., 2018). For example, foundational Cyanobacteria like *M. vaginatus* deters wind erosion (Kuske et al., 2012; Duniway et al., 2019) by rapidly proliferating filaments through unconsolidated surfaces ultimately increasing the threshold friction velocity of surface materials (Hu et al., 2002). Additionally, exopolymeric substance produced by cyanobacteria-dominated crust bacteria may enhances soil structure, improves infiltration (Costa et al., 2018), and may lower runoff and sediment loss due to increased soil aggregation (Faist et al., 2017). Alternatively, colonizing Cyanobacteria are often non-heterocystous incapable of performing N-fixation (Belnap, 1996; Yeager et al., 2007). Thus, once burgeoning Cyanobacteria-dominated biocrust communities mineralize and exhaust the residual unburned materials in soils (Prietofernandez et al., 1993), N availability may limit the ecosystem benefits of bacterial function.

In this study, we evaluated the potential for a burgeoning biocrust to recover and provide ecosystem services within a year following fire. We experimentally burned tracked the form and function of Cyanobacteria/lichen-dominated biocrusts and Cyanobacteria/moss-dominated biocrusts in a cold desert (UT, USA). Specifically, in a large-scale field manipulation, we burned the plant community and soil surfaces and evaluated shifts in bacterial community composition metrics such as richness, alpha diversity and taxa co-occurrence patterns 1 week, 2 months, and 1 year after the fire. We also measured N fixation rates and soil inorganic N availability, infiltration rates, and aggregate stability over the same time scale. The two biocrust forms occupied distinct locations across the landscape-Cyanobacteria/moss crust dominated the surfaces beneath and adjacent to canopies of *Artemisia tridentata* ssp. *Wyomingensis* shrub-islands, while Cyanobacteria/lichen crust dominated the shrub interspaces, which also supported a relatively low grass cover. We evaluated the cover of lichens, mosses, and surface cyanobacteria in burned and unburned plots. We hypothesized that, post-fire, a simplified Cyanobacteria-dominant crust will form from *M. vaginatus* and heterotrophic bacteria. We also hypothesized that bacterial biomass, richness, and diversity will approach unburned crust levels due to the resistance and resilience of soil bacteria to harsh conditions and high dispersal capabilities. Last, we hypothesized that within a year, along with a rudimentary crust, soil infiltration and stability, but not N-fixation, will begin to recover.

MATERIALS AND METHODS

Site Description

We conducted our study in the Great Basin Desert in Rush Valley, UT (40°05′27.43″N−112°18′18.24″W). Rugose crusts consisting of one moss, *Syntrichia caninervis* (9% cover) multiple cyanolichen and green algal lichen species (26% cover), and one Cyanobacterium, *M. vaginatus* (50% cover) were found in the shrub interspaces, while *S. caninervis* (6% cover) *M. vaginatus* (17% cover) and plant litter (70% cover) were found beneath shrubs. The shrub community was dominated by *A. tridentata,* ssp. *Wyomingensis*, and a native perennial grass *Elymus elymoides* (Raf.) Swezey. Mean annual precipitation (MAP) at the site is 27 cm year^{-1} [±1.5, $n = 30$, (mean and SEM) years 1978–2018] and mean annual temperature (MAT) is 8.8°C [±0.16, $n = 30$, years 1978–2018; Vernon Utah COOP Station 429133]. Based on limited climate data available from the station during our year-long experiment, the cold desert was slightly warmer and drier in the fall and winter than the 30-year mean. For example, monthly temperatures were within 1°C of MAT (months: October, November, August, September, and October), except in December when the mean daily temperatures was 3°C higher than MAT. Cumulative precipitation was a total of 2.5 cm lower in October, August, September, and October than the MAP levels for these same months. Soils were derived from Lake Bonneville sediments and are strongly alkaline. The series consists of well-drained, fine-loamy, mixed, mesic Xerollic Calciorthids with 3 to 15% calcium carbonate.

Fire Manipulation and Biocrust Locations

To investigate the post-fire response of biocrust bacteria, we created burned and unburned control plots. Treatments were assigned in a complete randomized block design for a total of 20 experimental plots (10 burned, 10 control plots), each 30 m width × 30 m length. Within each plot, we sampled two biocrust locations: Cyanobacteria/lichen-dominated crusts residing in interspaces ∼30 cm away from a shrub (interspace); and, Cyanobacteria/moss-dominated crusts beneath *A. tridentata* at the edge of the shrub canopy (shrub). We sampled plots and crust locations 1 week (27th September), 2 months (4th November), and 1 year (1st October) following the fire. To facilitate a thorough and even burn, straw was spread onto the soil surface before burning (Esque et al., 2010). Also, the ash from burned straw blew away from soil surfaces within days, reducing the likelihood of long-term increases of soil C substrates or nutrients from burnt straw.

Bacterial Biocrust Community Composition

To evaluate the effects of fire on Cyanobacteria and heterotrophic bacteria, we characterized bacterial community composition using target-metagenomics based on the 16S rRNA gene. Bacterial communities were evaluated from a composite surface soil sample from three subsamples (2 cm width × 2 mm depth) with a soil corer (2 fire treatments × 2 biocrust microsites × 3 time points × 3 replicates = 36 samples). Subsamples were: composited by biocrust location and treatment combinations within a plot, flash frozen in the field with liquid N, and stored at −20°C until DNA analysis We extracted genomic DNA from 0.5 g of soil using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA) and amplified the V4–V5 region of the 16S rRNA gene using the bacterial specific primer set 515F and 806R with a 12-nt error correcting Golay barcodes (Aanderud et al., 2019). We used the following thermal cycle for PCR reactions: an initial denaturation step at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 30 s, and an extension at 72°C for 90 s. The amplified DNA was purified (Agencourt AMPure XP PCR Purification, Beckman Coulter Inc., Brea, CA, USA), pooled at approximately equimolar concentrations (Quant-iT PicoGreen dsDNA Kit, Invitrogen Corporation, Carlsbad, CA, USA), and sequenced at the Brigham Young University DNA Sequencing Center (http://dnasc.byu.edu/) using a 454 Life Sciences Genome Sequence FLX (Roche, Branford, CT, USA). We analyzed all sequences using *mothur* (v. 1.29.2) to remove barcodes and short reads, chimeras, and non-bacterial sequences (Schloss et al., 2009). Specifically, we excluded sequences < 260 bp with homopolymers longer than 8 bp, removed chimeras using UCHIME (Edgar et al., 2011), and eliminated chloroplast, mitochondria, archaeal, and eukaryotic 16S rRNA gene sequences based on reference sequences from the Ribosomal Database Project (Cole et al., 2009). We then aligned sequences against the SILVA database (silva.nr_v128.align; Pruesse et al., 2007) with the SEED aligner and created operational taxonomic units (OTUs) based on uncorrected pairwise distances using a minimum coverage of 99% and minimum pairwise sequence similarity of 97%.

To analyze shifts in bacterial communities following the burn manipulation, first, we used Principal Coordinates Analysis (PCoA) and permutational multivariate analyses of variance (PERMANOVA, Anderson, 2001). The PCoA was based on a Bray-Curtis distance matrix using the "vegan" package in R (R Development Core Team, 2017). The PCoA aided in the visualization of communities, but we tested for the main effects and interactions between burn treatment and time since the fire with PERMANOVA using the *adonis* function also in the vegan package of R. Second, we calculated the relative recovery (i.e., relative abundance) of 10 phyla and three subclasses to identify differences in the distribution of major taxonomical groups (recovery ≥ 1.0%) among the burned and unburned biocrust types through time. Next to further evaluated shifts in bacterial communities, taxonomic trends of 20 families (recovery ≥ 1.0% in at least one replicate) were visualized in a heat map with hierarchal clustering using the *heatmap* function in the "gplot" package in R. Last, we quantified the alpha diversity of communities as the inverse Shannon index and richness as the total number of OTUs based on 1,000 iterations of 900 random resampled sequences from each replicate. We examined differences in alpha diversity and richness among fire treatments and biocrust locations through time using two-way, repeated measures ANOVA (RM-ANOVA) in R.

Biomass Estimates of Biocrust Bacteria

To evaluate the recovery of bacterial biomass in the burn manipulations, we estimated abundance of bacteria using

quantitative PCR and a universal bacterial 16S rRNA primer set [EUB 338 (forward) and Eub518 (reverse)] (Aanderud et al., 2018). In 12.5 µl reactions using KAPA2G Robust PCR Kits (KAPA Biosystems, Wilmington, MA, USA), we amplified targeted genes using the thermocycler condition: an initial denaturation step at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 45 s, anneal at 60°C for 30 s, and an extension at 72°C for 90 s. We generated qPCR standards from a crust soil bacterium using the TOPO TA Cloning Kit (Invitrogen) and extracted plasmids from transformed cells (Qiagen Sciences, Germantown, MD, USA). The coefficient of determination (r^2) for our assay was 0.98, while amplification efficiency was 1.5. We evaluated shifts in biomass with two-way, RM-ANOVA in R.

Network Co-occurrence Models of Biocrust Communities

To assess interactions among Cyanobacteria and other bacteria taxa, we created network co-occurrence models for the burned and unburned crust communities for each biocrust type based on maximal information coefficient (MIC) analysis. We calculated all possible linear and non-linear associations between OTUs using "Minerva" package in R, which belongs to a class of maximal information-based non-parametric exploration statistics for identifying and classifying relationships (Reshef et al., 2011). For all models, we included burned and unburned biocrust from the 2-month and 1-year sampling dates ($n = 6$ for each model), as the 1-week bacterial communities demonstrated little difference between the treatments in the PCoA. The nodes in the networks represented individual OTUs at 97% identity, while edges corresponded to valid or robust co-occurrence connections that occurred in at least 75% of all samples and had a MIC that was both > 0.7 and statistically significant (P-value $= 0.01$; Barberan et al., 2014). The filtering facilitated the determination of the core soil community responding to fire and removed poorly represented OTUs reducing network complexity. To describe the topology of the networks, we calculated the mean path length, mean degree, and modularity (Freedman et al., 2016). Network graphs in the *graphml* format were generated using "igraph" package in R and were visualized with Gephi (v. 0.8.2-beta).

Cyanobacteria/Lichen and Lichen Biocrust Community Composition

To describe changes on the soil surface, we used a sixteen-point grid and a modified, step point-intercept transect technique (Bowker et al., 2008) to estimate the mean percent ground cover of Cyanobacteria biocrusts; species of cyanolichen, green algal lichen, and mosses; bare ground; plant material covering soil surfaces, and rock in burned and unburned treatments. The grids were placed in fixed locations for each sampling time point to more accurately re-evaluate crust components through time. Cyanobacterial biocrust cover was estimated visually, light and dark surface coloration, and structurally by dropping a pin onto the soil surface to ensure that the crust was in fact a crust created by cyanobacterial colonies weaving through soil surfaces (Rosentreter et al., 2007).

Biocrust N Fixation and Inorganic N

To determine the effects of fire on soil N inputs, we measured N fixation (µmol h^{-1} m^{-2}) using the acetylene reduction assay (ARA) and evaluated soil ammonium (mg N-NH_4^+ kg $soil^{-1}$). The ARA assay was measured in intact soil core (2 cm width × 1 cm depth), while inorganic N was assessed from a composite surface soil sample from three subsamples (2 cm width × 2 mm depth) with a soil corer. Both N determinations were evaluated on all 10 field replicates through time (2 fire treatments × 2 biocrust microsites × 3 time points × 10 replicates = 120 samples). For ARA, we followed the protocols outlined by Belnap (2002). Briefly, we: incubated cores for 2 days on a 12 h light, 12 h dark schedule with daily water additions of 1 ml; sealed the cores and created a 10% acetylene atmosphere in the headspace by injecting 5 ml of pure acetylene through a septum with a gas-tight syringe; 4 h later, we removed a 4 ml headspace gas sample and measured the concentration (ppm) of ethylene with an Agilent Technologies 6890A gas chromatograph with a PoraPak R column (Agilent Technologies, Santa Clara, CA, USA) with an attached flame ionization detector. We used the ideal gas law to convert ppm ethylene to µmol ethylene h^{-1} m^{-2}. We measured ammonium in soil extracts (2 g soil) with 4 ml 0.5 M K_2SO_4 (1:2 w/v) and quantified the N-NH_4^+ using a SpectraMax Plus 384 (Molecular Devices Corporation, Sunnyside, CA, USA; Miranda et al., 2001). We tested for the effects of the fire treatment and biocrusts location on N fixation through time using two-way, RM-ANOVA.

Infiltration Rates and Soil Aggregate Stability

To investigate the effect of fire on soil infiltration rates, we measured changes in soil infiltration rates (cm s^{-1}) with a Decagon Device's Mini Disk Tension Infiltrometer (METER Group, Pullman, WA) and calculated infiltration following the method outlined by Zhang (1997). Due to the rugose nature of the Cyanobacteria/moss-dominated biocrusts at this site, we added ∼10 g of quartz sand to help the infiltrometer form a seal with the soil surface. To assess soil aggregate stability, we measured soil aggregate (6–8 mm in diameter) stability according to the Jornada Experimental Range Test (Herrick et al., 2001) with a kit designed by Synergy Resource Solutions, Inc. (Montana, USA). Briefly, aggregates were assigned to a stability class (1–6) based on a combination of visual observation of slaking following the immersion of the aggregate in distilled water and the percent aggregate remaining on a 1.5 mm sieve after five dipping cycles. We tested for treatment effects though time using two-way, RM-ANOVA.

RESULTS

Fire Impact on Biocrusts

The impact of fire on Cyanobacteria/lichen- and Cyanobacteria/moss-dominant crusts was dramatic, with no visual recovery of biocrusts on burned soil surfaces during any of our sample times. None of the Cyanobacteria (light or dark surface coloration or surface structure), cyanolichens, green algal lichens, or mosses that composed the visible portion of

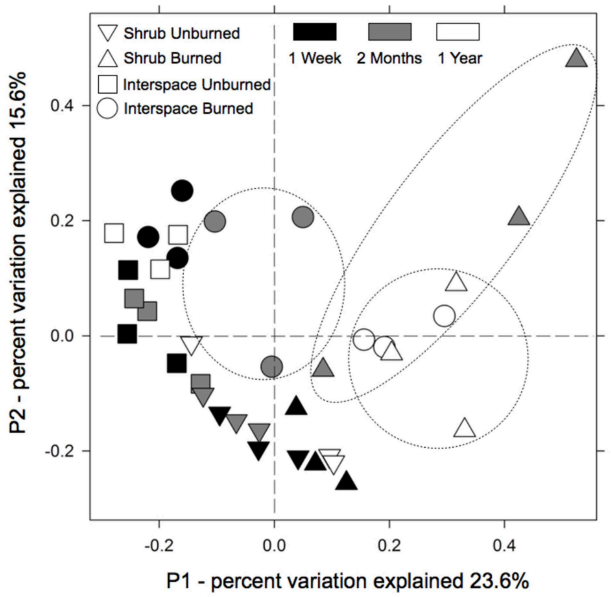

FIGURE 1 | Bacterial communities in burned Cyanobacteria/lichen-dominated (interspaces) and Cyanobacteria/moss-dominated (shrubs) biocrusts converged to a common composition 1 year after the fire. The multivariate ordination was generated using principle coordinate analysis (PCoA) on a sample × OTU matrix of 16S rRNA gene community libraries (97% similarity cut-off).

biocrusts recovered in a year (**Supplemental Table 1**). One year after the fire, bare soils covered 93% ± 5.1 of the interspaces and 86% ± 5.4 of the surfaces beneath shrub canopies, with a sparse cover of perennial grass, *E. elymoides*, annual exotic grass, *Bromus tectorum* (interspace = 0; shrub = 3.8% ± 3.8) and/or the noxious exotic annual forb *Halogeton glomeratus* (interspace = 1.9% ± 1.9; shrub = 3.1% ± 1.4).

One year after the fire, burned soil biocrusts eroded away with the wind. Burned mosses and lichens remained attached to soil surfaces 2-months after the fire (data not shown); however, after 1 year all that remained was bare soil. Based on the new exposure of burned *A. tridentata* stems 1-year after the fire and the barren surfaces denuded of moss and lichen cover, anywhere from 0 to 2 cm of biocrusts constituents and some soil potentially eroded away. The unburned biocrusts remained intact over the year. Cyanobacteria/lichen crusts were dominated by one cyanolichen, *Collema tenax* (16% ± 3.5), and a green algal lichen, *Toninia sedifolia* (3.1% ± 1.4).

Fire Caused a Convergence of Biocrust Bacterial Communities

A year after the fire, the burned biocrusts, regardless of type, converged to a common bacterial community. The PCoA results clearly separated burned from unburned communities in ordination space along axis 1, which explained 23.6% of the variation (**Figure 1**). By the end of the year, the soil communities residing in the now mostly barren soils of burned interspace Cyanobacteria/lichen- and shrub Cyanobacteria/moss-dominated crusts were similar and grouped together. Conversely, unburned communities retained a signature of their biocrust type in ordination space along axis 2 (15.6% of the variation) with Cyanobacteria/lichen- and cyanobacteria/moss-dominated crusts creating unique bacterial communities. PERMANOVA results supported the ordination; there was a fire treatment × time interaction on bacterial composition (PERMANOVA, $F = 2.39$, $R^2 = 0.09$, $P = 0.001$), suggesting that fire effect persisted even 1 year, with limited recovery. Also, there was a fire treatment × location on bacterial community composition (PERMANOVA, $F = 2.00$, $R^2 = 0.04$, $P = 0.02$), demonstrating that fire altered bacterial communities differently in two biocrust types. This interaction was highlighted as 2-month-old, burned shrub communities were the first to shift from its unburned crust counterpart. All community inferences were based on the recovery of 127,616 quality sequences and 3,345 unique OTUs with samples possessing an average sequencing coverage of 89% ± 0.004 (mean and SEM) and normalized to 3,345 sequences. All sequences are available through NCBI as BioProject SUB6427289.

Dominant Cyanobacteria Replaced by Rare Firmicutes Following Fire

Across both biocrusts, fire caused one dominant phylum to disappear and another to appear. Beneath shrubs, the abundance of Cyanobacteria was 72-times lower in the burned (0.12% ± 0.11) than unburned treatment (8.4% ± 2.6) 2 months after the fire, and after 1 year, was undetectable (**Figure 2**). Similarly, in interspaces, Cyanobacterial abundance was thirteen-times lower in the burned (1.1% ± 0.31) than unburned treatment (14% ± 5.2) 2 months post-fire, and after 1 year, was only barely detectable in burned soils (0.01% ± 0.01). Within the Cyanobacteria, the family Microcoleaceae, more specifically *M. vaginatus*, was the foundational Cyanobacterial taxon, constituting 13% (± 2.7) of bacterial abundance in Cyanobacteria/lichen crusts and 4.7% (± 1.4) in Cyanobacteria/moss crusts, regardless of sampling time (**Figure 3**). *Nostoc* and *Chrooccoccidiopsis* species also occurred in crusts but their abundance rarely exceeded 1% relative recovery. The resulting bacterial gap in burned biocrusts was filled by rare Firmicutes (**Figure 2**). Firmicutes were not detectable in unburned Cyanobacteria/lichen and only barely present in Cyanobacterial/moss biocrusts (abundance across all time points = 0.3% ± 0.2); however, 2 months following the fire, Firmicutes constituted 12% ± 5.2 of burned interspace bacteria and 35% ± 16 of burned shrub communities' bacteria. One year after the fire, Firmicutes remained a dominant phylum in burned interspaces (13% ± 5.8) and beneath burned shrubs (9.1% ± 6.0). The Planococcaceae, specifically a *Planococcus* species, within the Firmicutes differentiated the two burned community types and dominated burnt soils (**Figure 3**). The recovery of the Planococcaceae in the 2-month- and 1-year-old burnt communities = 12% ± 3.5 in interspace and 19% ± 7.5 in shrub crusts).

Other heterotrophic bacteria distinguished burned from unburned biocrusts, especially families from the Actinobacteria (**Figure 3**). Burned soil conditions enhanced the recovery

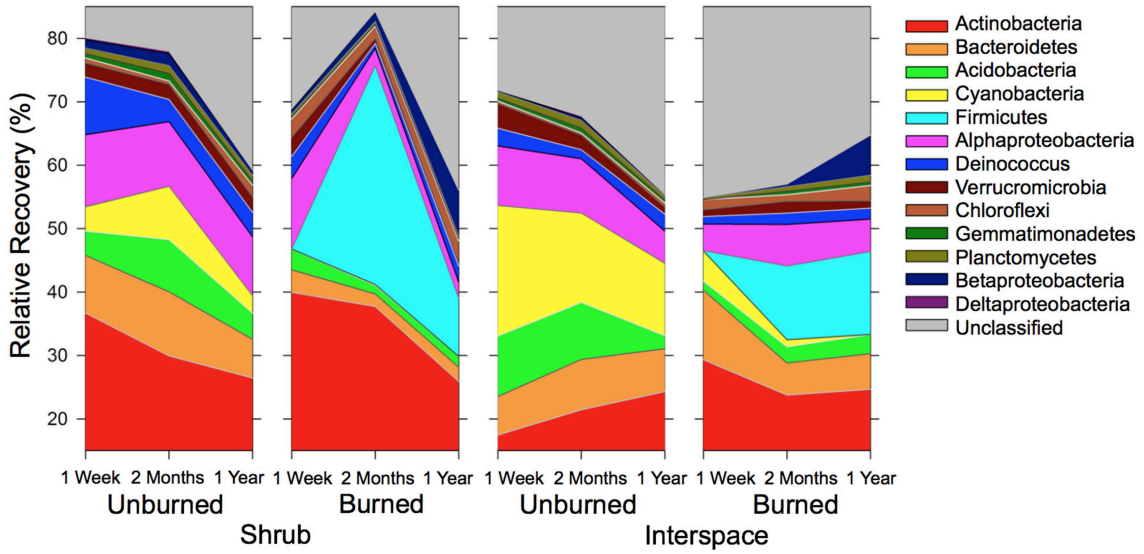

FIGURE 2 | Abundant Cyanobacteria were replaced by rare Firmicutes in burnt crusts. The distribution of OTUs (%) is presented for the 10 phyla and three Proteobacteria subclasses that contributed ≥ 1% to the total recovery of biocrust communities. Values are means ($n = 3$) based on 16S rRNA gene libraries.

of the Cellulomonadaceae (Actinobacteria), which was at least 9.0-times higher in burned than unburned biocrusts, with recovery reaching 8.8% ± 3.1 and 8.3% ± 4.6 in 1-year-old burned and now bare interspace and shrub soils respectively. The Oxalobacteraceae (Betaproteobacteria) also dominated burnt soils after 1 year, with abundance increasing from 0.11% (± 0.09) in unburned to 5.7% (± 1.6) burned interspace and 0.33% (± 0.09) in unburned to 6.9% (± 4.1) burned shrub soils. One-year post-fire, the abundance of the Micromonosporaceae (Actinobacteria), Rubrobacteriaceae (Actinobacteria), and Chitinophagaceae (Bacteroidetes) was at least 1.8-times lower in bare soils than unburned biocrusts, regardless of type. In Cyanobacteria/moss-dominated biocrusts the abundance of Sphingomonadaceae (Alphaproteobacteria) was depressed 4.2-fold one year after fire.

Biomass Recovered While Richness and Diversity Remained Unchanged Post-fire

Bacterial biomass in the bare surface soils recovered 1 year after the fire, in contrast to richness or diversity that remained relatively stable. In burned shrub soils, bacterial biomass (16S rRNA gene copy number) declined an order of magnitude 1 week, and two orders of magnitude 2-months post-fire but recovered after 1 year (two-way RM-ANOVA, interaction: fire × time, $F = 296$, $P < 0.004$, $df = 2$, **Figure 4**). In interspace soils, bacterial gene copy numbers were consistently lower in burned than unburned cyanobacteria/lichen-dominated biocrusts. In both crust types, OTU richness in all biocrusts and burnt soils was lowest 1 week (273 ± 34.7), highest 2 month (461 ± 27.51), and moderate 1 year (357 ± 13.5) following the fire (two-way RM-ANOVA, main effect: time, $F = 6.9$, $P = 0.004$, $df = 2$, data not shown). No trend was visible for diversity, which ranged from 2.7 (± 0.77) in 2-month, burnt shrub to 4.2 (± 0.05) in 2-month, unburned shrub crusts (data not shown).

Fire Deconstructed Biocrust Bacterial Communities

Fire reduced the network complexity and connectedness present in Cyanobacteria/lichen- and Cyanobacteria/moss-dominated biocrusts. For example, network models for both burned compared to unburned biocrust types contained: 40–75% reduction in the number of significant correlations or edges between nodes or OTUs, up to a 57% increase in mean path length (number of steps between each node and any other node), and at least a 1.4-times smaller mean degree (average number of edges connected to a node, **Figure 5**, **Table 1**). Within the network models, 70–80% of the "hub" OTUs or the top 10 highest connected nodes (**Table 1**) were different between burned and unburned biocrusts. Species belonging to the Acidobacteriaceae (Acidobacteria), Chloroflexaceae, Thermomicrobia (Chloroflexi), and Gemmatimonadaceae (Gemmatimonadetes), were unique hubs in burned shrub soils, while species from the Acidobacteriaceae (Acidobacteria), Acidomicrobineae, Microbacteriaceae Micrococcacaceae, Micromonosporaceae, Solirubrobacteriaceae (Actinobacteria), Chitinophagaceae (Bacteroidetes), and Trueperaceae (Deinococcus) were unique hubs in burned interspace soils (**Supplemental Table 2**).

M. vaginatus (Cyanobacteria) was present in both unburned models; however, it was not a hub species. The Firmicutes that dominated communities following fire failed to influence any OTU in the burned interspace model and was only slightly connected to several other nodes in burned shrub model.

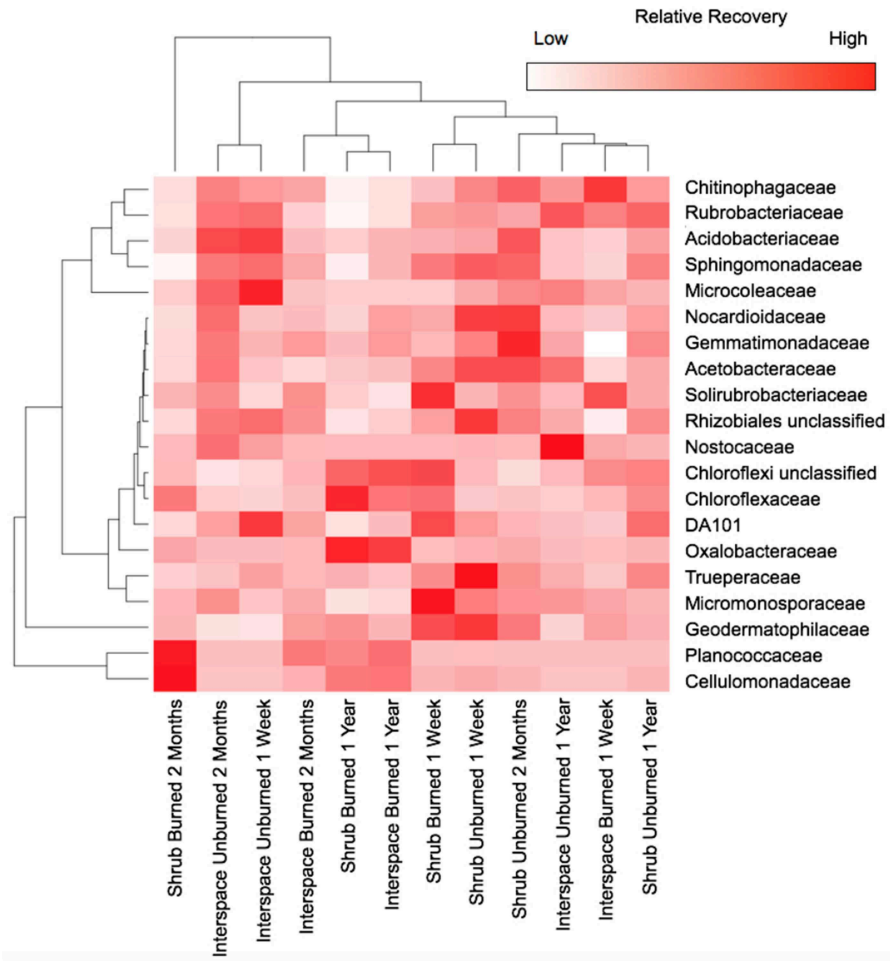

FIGURE 3 | Burning and time since fire selected for specific bacterial families based on heat map analysis and hierarchical clustering of the relative recovery of 20 bacterial families. Values are based on means with hierarchal clustering of the burned treatments, and shrub and interspace microsites (top) and families (left). Only families that contributed ≥ 1.0% to the total recovery of communities are presented with recovery based on 16S rRNA gene libraries. Values are means ($n = 3$).

Fire Depressed N Fixation in Cyanobacteria/Lichen-Dominated Biocrusts

Fire reduced the capacity of Cyanobacteria/lichen-dominated biocrusts to fix N, but inorganic N did accumulate in all burned crusts over time. In interspaces, N fixation rates were 6-times and thirty-one-times lower in burned than unburned treatments 1 week and 2 months after the fire, respectively (RM-ANOVA, interaction: fire × microsite × time, $F = 10.4$, $P < 0.0001$, $df = 2$, **Figure 6A**). Interspace N fixation rates were still depressed 1 year after the fire, being 3.5-times lower in burned than unburned cyanobacteria/lichen-dominated biocrusts. N fixation rates in all cyanobacteria/moss-dominated crusts were consistently low regardless of the fire. Fire increased N-NH_4^+ concentrations in both burned biocrust types over the 1-year experiment (RM-ANOVA, interaction: fire × time, $F = 60$, $P < 0.0001$, $df = 2$) with N-NH_4^+ concentrations slightly higher in interspace than shrub surface soils (RM-ANOVA interaction: microsites × time, $F = 3.7$, $P = 0.03$, $df = 2$, **Figure 6B**).

Fire Depressed Infiltration Rates While Soil Aggregate Stability Was Insensitive

Fire depressed soil infiltration rates, especially shortly after the burn. Although infiltration was variable in unburned crusts, infiltration rates were at least 5.5-times lower in burned than unburned interspace and shrub soils 1 week after the fire (RM-ANOVA, interspace, fire × time, $F = 31$, $P < 0.0001$, $df = 2$, **Figure 7**). During the remainder of the year, infiltration rates in both biocrust types were only slightly depressed, at most 1.5-fold. Soil stability was barely impacted by fire, as stability was only depressed at the 1-week sample time in burned under-shrub Cyanobacteria/moss biocrusts (4.0 ± 0.70) compared to control treatment (7.1 ± 0.67, RM-ANOVA, interaction: fire × time, $F = 3.0$, $P < 0.05$, $df = 2$, data not shown).

DISCUSSION

Following fire, surviving taxa, colonizing species from soils immediately below the burn, and/or pioneering bacteria attached

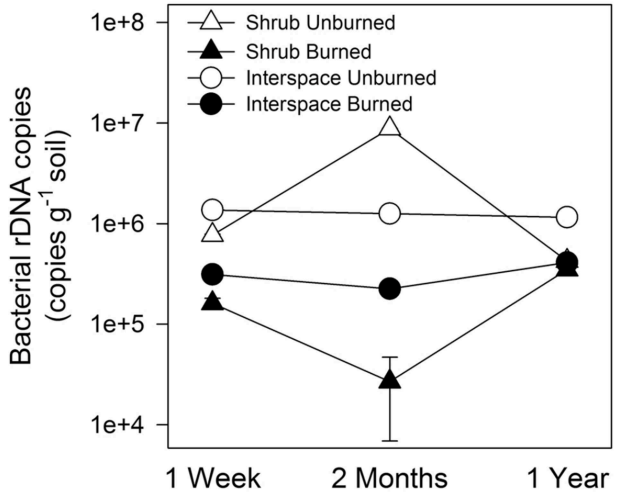

FIGURE 4 | Bacterial biomass declined following fire in both biocrust types. Values are means (n = 2–3) ± SEM, measured with quantitative polymerase chain reaction (qPCR) and reported as rRNA gene copy number. Differences between burned and unburned soils through time are based on two-way, repeated-measures RM-ANOVA.

to bioaerosols may rapidly create unique communities in multiple ecosystems (Williams et al., 2012; Ferrenberg et al., 2013; Xiang et al., 2014; Li et al., 2019). Our cold desert was no different. Although aspects of the community (i.e., bacterial biomass, alpha diversity, and richness) recovered in the short-term, we found no evidence of the burned bacterial community becoming a burgeoning biocrust. After 1-year, the fire facilitated the formation of a unique convergent community of chemoheterotrophic copiotrophs in the resulting bare surface soils that were once the burnt shrub and interspace biocrusts. The emergent community still helped glue soil aggregates together, but N fixation and soil infiltration were depressed. Taken together, the loss of much of the photosynthetic and N_2 fixation potential due the disappearance of Cyanobacteria and/or higher plants calls into question the longevity of the emergent community. The burgeoning community of chemoheterotrophic copiotrophs may only persist until the C and N released from the burn are exhausted. Our results demonstrate that biocrust bacteria may recover rapidly after fire, albeit along a different trajectory that results in fewer ecosystem services.

Dominant Foundational Cyanobacteria Disappeared After Fire

Contrary to our hypothesis, a simplified Cyanobacteria crust failed to form. *M. vaginatus*, the foundational Cyanobacteria in our unburned biocrusts, disappeared or was barely detectable in burned soils after 1 year. *M. vaginatus* did persist 2 months after the fire as an active member, dormant cell, or persistent exogenous DNA. The loss of Cyanobacteria was accompanied by a reduction in chemoheterotrophic copiotrophs, often associated with the "cyanosphere" (Couradeau et al., 2019). The cyanosphere is a nutrient-rich zone, analogous to the rhizosphere, where cyanobacteria enhance fertility of surface soils, primarily through increasing organic C availability via the addition of photosynthate, inorganic N through fixation, and soil moisture due to the specific ecohydrological benefits of crusts. Taxa from three of our families, in particular, were abundant in our unburned cyanosphere and likewise major components of other Cyanobacteria-, lichen-, and/or bryophyte-dominated crusts, Rubrobacteriaceae (Actinobacteria, Nagy et al., 2005; Gundlapally and Garcia-Pichel, 2006; Angel and Conrad, 2013; Maier et al., 2018), Chitinophagaceae (Bacteroidetes, Kuske et al., 2012; Maier et al., 2018), and Sphingomonadaceae (Alphaproteobacteria, Maier et al., 2014). Our sampling technique did not explicitly identify taxa in direct contact with Cyanobacterial filaments but captured taxa within the cyanosphere and in soil immediate surrounding the filaments. Even with our more course sampling, the cyanosphere and other biocrust constituents seem to generate a predictable set of soil conditions that may favor specific bacterial taxa.

Rare Firmicutes Dominated Burnt Heterotrophic Communities

The emergent community was dominated by a previously rare *Planococcus* species (family Planococcaceae, Firmicutes) that dominated all burned soils. In general, Firmicutes are favored in soils following fire, especially in the short-term (Ferrenberg et al., 2013). Our *Planococcus* species was no exception. This species appears able to occupy, rapidly populate, and dominate the same location as biocrusts constituents (the uppermost millimeters of soil), perhaps by exploiting nutrient-rich shifts in soils induced by fire. *Planococcus* are moderately halophilic heterotrophic (Ventosa et al., 1998) present in cold deserts around the world capable of hydrolyzing starch (Reddy et al., 2002; Mayilraj et al., 2005). In this instance, *Planococcus* appeared able to rapidly utilize relatively labile starches released after the burn fire without influencing other taxa, as this species was not a hub species in any network or only slightly connected to several other taxa in the shrub model network. Thus, *Planococcus* are most likely a copiotroph scrambler (Hibbing et al., 2010), better suited to capitalize on emerging resources by scrambling for nutrients instead of contesting/competing for existing ones. Other copiotrophs dominated burned soils, specifically a *Cellulomonas* species in the Cellulomonadaceae (Actinobacteria) and *Massilia* species in the Oxalobacteraceae. Both species were also not hub species, but may capitalized on partially burned plant/algal materials remaining in soils. The *Massilia* genera houses facultative anaerobes that are able to degrade long chain hydrocarbons in oil contaminated soils (Ali et al., 2016; Ren et al., 2018) and reduce nitrate in biocrusts (Bailey et al., 2014). The *Cellulomonas* generate houses Gram-positive, aerobic bacteria able to degrade cellulose (Anderson et al., 2012). The emerging dominant bacteria in our converged desert communities were presumably copiotrophs utilizing C resources released after fire. Once the partially burned and available organic C sources are consumed, we project that the community will shift once again, especially if there are no new inputs of C from photosynthetic organisms.

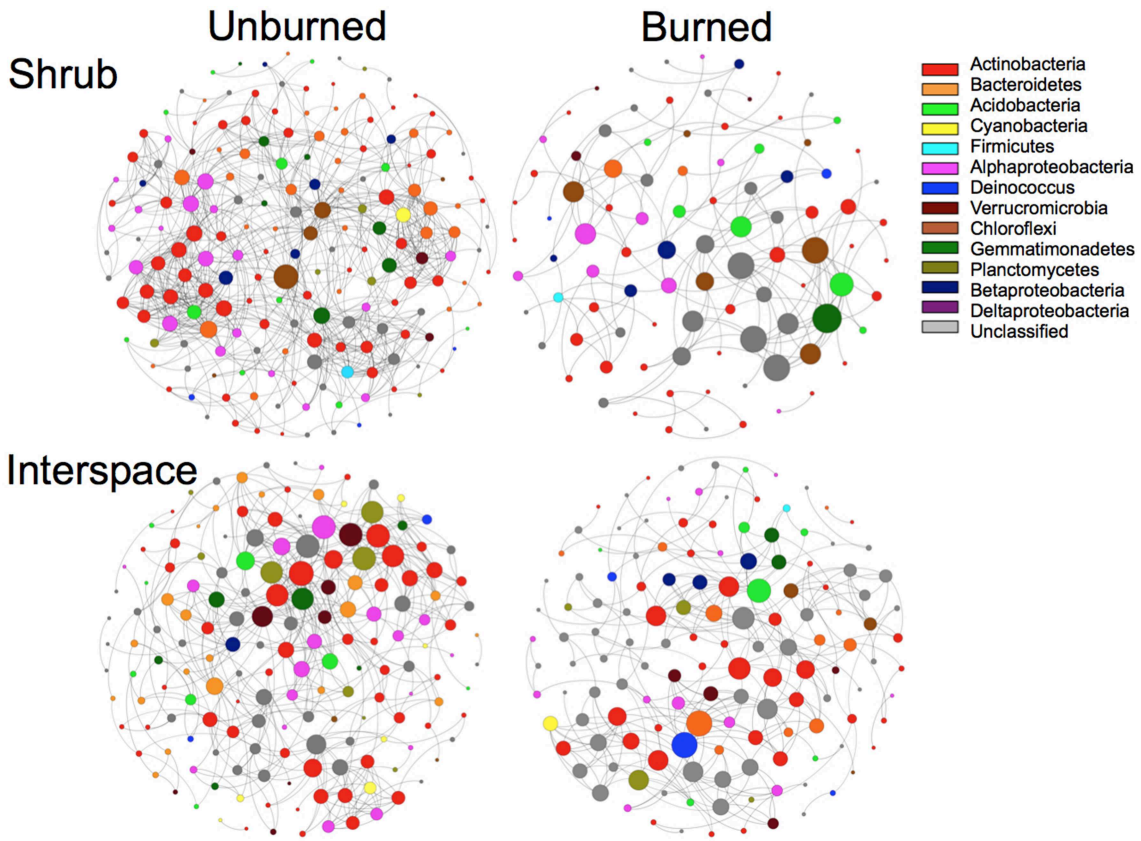

FIGURE 5 | Network co-occurrence models using maximal information coefficient (MIC) analysis of unburned and burned biocrusts after fire. Nodes (circles) in the network models represent OTUs from 16S rRNA gene community libraries (97% similarity cut-off) with edges (connecting lines between nodes) representing significant co-occurrence connections that occur in at least 75% of the samples for a given microsite and have an MIC that is both > 0.7 and statistically significant ($P < 0.01$, $n = 6$). Colors represent different phyla as shown in the figure key. The size of the nodes indicates the number of significant connections between the given node and other taxa.

TABLE 1 | Metrics from network co-occurrence models of burned and unburned of Cyanobacteria/lichen-dominated crusts occupying relatively plant-barren interspaces (interspaces) and Cyanobacteria/moss-dominated crusts the beneath shrub-islands (shrub).

	Shrub		Interspace	
Metrics	**Unburned**	**Burned**	**Unburned**	**Burned**
Nodes	148	95	167	140
Edges	614	152	514	307
Mean path length	3.5	5.5	4.3	5.2
Mean degree	8.3	3.2	6.2	4.4
Modularity	0.61	0.76	0.69	0.71

Firmicutes are often only minor constituents in biocrusts but may become more abundant in disturbed biocrusts. In multiple metagenomic surveys of biocrust communities from hot and cold deserts, Firmicutes are relatively uncommon (Steven et al., 2014; Karaoz et al., 2018; Warren et al., 2019). Following multiple forms of disturbance however, these aerobic, desiccation-tolerant copiotrophs may dominate. For example, following rewetting in Cyanobacteria-dominated biocrusts, *Microcoleus* species were displaced by large blooms of Firmicutes from three families (i.e., Alicyclobacillaceae, Bacillaceae, and Planococcaceae) one of which, the with Planococcaceae houses our *Planococcus* species (Karaoz et al., 2018). Further, once Cyanobacterial-dominated crusts are disturbed due to grazing, bacterial communities in now bare soils associated with hoofprints contain a high contribution of Firmicutes (Abed et al., 2019). Firmicutes are often spore-formers and the ability of these taxa to weather adverse environmental conditions as endospores most likely contributes to their success in disturbed biocrusts. Our dominant Firmicutes, a *Planococcus* species, however, is from a non-spore forming genera. The ability of the *Planococcus* to exploit disturbed biocrusts may reside in their protein flexibility, resource efficiency, genomic plasticity, and osmotic-specific adaptive mechanisms that likely compensate for the desiccation and cold stresses present in cold deserts (Mykytczuk et al., 2013).

Recovered Burned Communities Poorly Interconnected

As hypothesized, bacterial biomass, richness, and diversity recovered to approximately unburned levels 1 year after the fire even in bare soils, but the resulting community was more diffuse

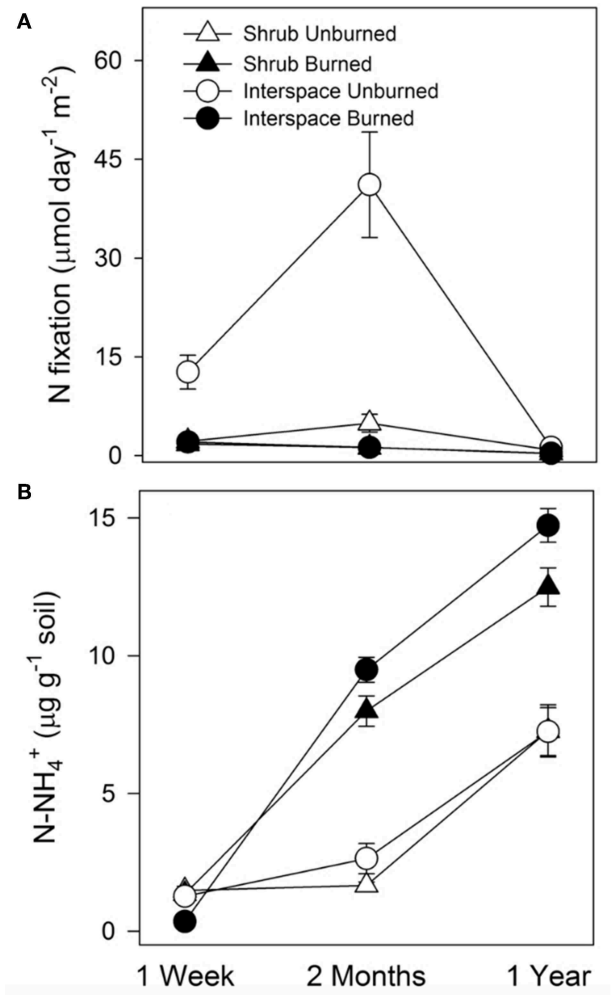

FIGURE 6 | N fixation rates **(A)**, and ammonium concentrations ($N\text{-}NH_4^+$) **(B)** in unburned and burned biocrusts following fire. N fixation ($\mu mol\ day^{-1}\ m^{-2}$) and inorganic N ($\mu g\ N\text{-}NH_4^+\ g^{-1}$ soil) values are means ($n = 10$) ± SEM. Differences between treatments are based on RM-ANOVA results from three time intervals following fire (1-week, 1-month, and 1-year).

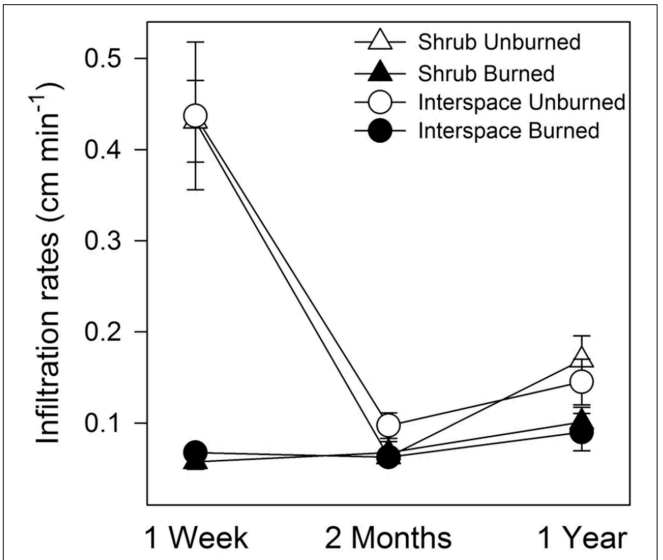

FIGURE 7 | Soil infiltration rates through biocrusts declined after fire. Values ($cm\ min^{-1}$) are means ($n = 5$), ± SEM, reported in $cm\ s^{-1}$. Differences are based on RM-ANOVA with attending F statistics and *P*-values for significant interactions.

and sparsely interconnected. In soils, disturbances that alter the quantity and quality of C and other resources may exert immense control over bacterial communities and microbial-mediated processes (Ma et al., 2015; Zechmeister-Boltenstern et al., 2015). With desert wildfires may come a flush of resources (Fuentes-Ramirez et al., 2015). Depending on fire severity, partially burnt plant materials deposited on soil surfaces and/or leached into the profile, along with defunct root systems, offers copious amounts of relatively labile and recalcitrant C for bacteria to harness. Additionally, fire may release phosphorus and other non-combustible nutrient potentially alleviating nutrient limitations and the availability of water may rise in the absence of transpiration by higher plants. In deserts, soils surrounding burned *A. tridentata* supported higher levels of bacterial biomass and increased concentrations of total organic C, total N, and dissolved organic C (Halvorson et al., 1997). Thus, burned soils may offer relatively nutrient-rich soil conditions and/or new niches for recovering bacteria to exploit. The dominant *Planococcus* species (Firmicutes) was not one of the top-ten most interconnected bacterial species in burnt soils. Fire created a unique set of interconnected hub species of chemoheterotrophic copiotrophs commonly found in biocrusts and/or other taxa well-adapted to weather and thrive in desert soil conditions. For example, new hubs, Acidobacteriaceae (Acidobacteria), Solirubrobacteriaceae (Actinobacteria), Chitinophagaceae (Bacteroidetes), are common copiotrophs within biocrusts (Kuske et al., 2012; Angel and Conrad, 2013; Maier et al., 2018) and potentially contribute/influence a consortium of taxa consuming cellulose, hemicellulose, and chitin within burnt soils. The Chloroflexi commonly associated with *M. vaginatus* in biocrusts are thermotolerant and non-photosynthetic scavenging organic acids derived from Cyanobacterial photosynthates (Maier et al., 2018). In burned soils beneath shrubs, the hub members of the Chloroflexaceae and Thermomicrobia were most likely thermophilic and cellulolytic taxa demonstrating photoheterotrophic and/or chemoheterotrophic metabolism (Houghton et al., 2015; Klatt et al., 2015). Thus, both hubs were possibly reliant or linked to other taxa generating organic acids in burned soils. Last, the Actinobacteria are desert cosmopolitan species, hosting taxa that are acidtolerant, alkalitolerant, psychrotolerant, thermotolerant, and halotolerant (Mohammadipanah and Wink, 2016), and able to produce dormant endospores under harsh conditions. Thus, we are assuming that our Gram-positive Actinobacteria hubs (i.e., Acidomicrobineae, Microbacteriaceae Micrococcacaceae, and Micromonosporaceae) were extremely well-adapted to weather the environmental extremes often present in

deserts and potentially compete for resources under burned soil conditions.

Recovering Community Provided an Abbreviated Set of Ecosystem Services

Our last hypothesis was predicated on the services provided by a rudimentary *M. vaginatus*-dominated crust. In the absence of Cyanobacteria; however, the recovered bacterial community provided only an abbreviated set of ecosystem services. Soil aggregate stability was only marginally impacted by the burn, dipping slightly in Cyanobacteria/moss-dominated crusts 2 months after the fire. Bacteria commonly produce exopolymeric substances that glue soil particles together (Costa et al., 2018). Our data suggest that the post-fire soil community aggregated soils into particles or at least helped maintain aggregation in now surface soils as proficiently as either biocrust type. The benefits of soil aggregation by biota are substantial (Bronick and Lal, 2005), but the emerging soil communities we observed after fire are unlikely able to withstand the high erosional forces of desert winds. Well-developed biocrusts, those with high biomass of crust constituents, reduce soil erodibility, and armor soils against wind erosion (Belnap and Gardner, 1993; Mazor et al., 1996). Our burned soils support similar amounts of bacterial biomass but the surfaces are void of crusts. Thus, substantial erosion is likely, regardless of soil aggregate stability. Alternatively, both infiltration and N fixation failed to recover following fire. We found some evidence of a hydrophobic layer forming on the burned soil surfaces, as infiltration plummeted 1-week after the fire and remained depressed through the year. We expect that the burning of biocrusts, *A. tridentata*, or the straw that we added to carry the fire and released hydrophobic organic compounds or rearranged amphiphilic molecules (e.g., phytanols and fatty acids) already present in the soil (Ravi et al., 2007; Uddin et al., 2017). N fixation was basically non-existent in burned soils, but soil ammonium continued to accumulate in burned surface soils, suggesting that the emergent community had adequate access to N.

Bromus tectorum and *Halogeton glomeratus* Invaded Burnt Biocrusts

Our burnt soils will most likely never recover into a fully-developed biocrust. Although the burnt soils were only sparsely covered with exotic, annual grass *B. tectorum* and noxious invader *H. glomeratus* 1 year after the fire, both species had fully encroached into the disturbed soils after only 4 years (St. Clair et al., 2016). Cover of *B. tectorum* increased from 3.8% (± 5.2) to 23% (± 5.2) and *H. glomeratus* increased from 0.9% (± 1.1) to 13% (± 1.1) in unburned compared to burned plots. Fully formed biocrusts often inhibit exotic but not native plant establishment (Slate et al., 2019), but disturbance may suppress this process (Hernandez and Sandquist, 2011).

Such invasion results in a greater percentage of vascular plant cover and subsequent litter on the soil surface, both of which reduce light levels reaching the soil surface, effectively threatening essential phototrophic component of crusts (Brooks and Matchett, 2006). Additionally, increased plant cover may compete for essential nutrients (e.g., N and phosphorus) and soil moisture necessary for crust recovery (Evans et al., 2001; Ryel et al., 2010). Thus, if fire occur in close proximity to exotic plant seed sources and the disturbed soils are readily invaded, the probability of a fully-developed crust is unlikely.

CONCLUSION

An intensive wildfire inhibited the recovery of even a rudimentary biocrust in 1 year's time. Although wildfires changed biocrusts into bare soils with vastly different community composition, both bacterial communities supported similar level of bacterial biomass, alpha diversity, and richness 1 year after the fire. Rather than being dominated by the Cyanobacterium *M. vaginatus*, the two burned biocrust types converged to a common community dominated by heterotrophic copiotrophs most likely benefiting from the release of partially burned biocrust and plant materials. The fire created more diffuse and poorly connected communities than their unburned biocrust counterparts disrupting upwards of 75% of species interactions present in unburned crusts. One common ecosystem service supported by biocrusts potentially returned (i.e., exopolymeric substances gluing soil aggregates together); however, the seminal biocrust services of N fixation and improved soil ecohydrology, measured here as soil infiltration rates, remained substantially reduced 1 year after the burn. Our results suggest that the absence of the dominant and foundational taxa of biocrusts opened multiple new niches for rare bacteria to exploit creating poorly connected communities that provided only an abbreviated set of ecosystem services.

DATA AVAILABILITY STATEMENT

This manuscript contains previously unpublished data. All sequences are available through NCBI as BioProject SUB6427289.

AUTHOR CONTRIBUTIONS

ZA, JBa, DR, JBe, TC, RG, BM, and SC conducted the experiments and helped write and review the manuscript. ZA, JBa, DR, JBe, and SC analyzed and interpreted the data. ZA agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00467/full#supplementary-material

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Neglected but Potent Dry Forest Players: Ecological Role and Ecosystem Service Provision of Biological Soil Crusts in the Human-Modified Caatinga

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Biological soil crusts (biocrusts) have been recognized as key ecological players in arid and semiarid regions at both local and global scales. They are important biodiversity components, provide critical ecosystem services, and strongly influence soil-plant relationships, and successional trajectories via facilitative, competitive, and edaphic engineering effects. Despite these important ecological roles, very little is known about biocrusts in seasonally dry tropical forests. Here we present a first baseline study on biocrust cover and ecosystem service provision in a human-modified landscape of the Brazilian Caatinga, South America's largest tropical dry forest. More specifically, we explored (1) across a network of 34 0.1 ha permanent plots the impact of disturbance, soil, precipitation, and vegetation-related parameters on biocrust cover in different stages of forest regeneration, and (2) the effect of disturbance on species composition, growth and soil organic carbon sequestration comparing early and late successional communities in two case study sites at opposite ends of the disturbance gradient. Our findings revealed that biocrusts are a conspicuous component of the Caatinga ecosystem with at least 50 different taxa of cyanobacteria, algae, lichens and bryophytes (cyanobacteria and bryophytes dominating) covering nearly 10% of the total land surface and doubling soil organic carbon content relative to bare topsoil. High litter cover, high disturbance by goats, and low soil compaction were the leading drivers for reduced biocrust cover, while precipitation was not associated Second-growth forests supported anequally spaced biocrust cover, while in old-growth-forests biocrust cover was patchy. Disturbance reduced biocrust growth by two thirds and carbon sequestration by half. In synthesis, biocrusts increase soil organic carbon (SOC) in dry forests and as they double the SOC content in disturbed areas, may be capable of counterbalancing disturbance-induced soil degradation in this ecosystem. As they fix and fertilize depauperated soils, they may play a substantial role in vegetation regeneration in the human-modified Caatinga, and may have an extended ecological role due to the

ever-increasing human encroachment on natural landscapes. Even though biocrusts benefit from human presence in dry forests, high levels of anthropogenic disturbance could threaten biocrust-provided ecosystem services, and call for further, in-depth studies to elucidate the underlying mechanisms.

Keywords: biological soil crusts, Caatinga, dry forest, exotic goats, human disturbances, soil organic carbon

INTRODUCTION

Biological soil crusts (hereafter referred to as "biocrusts") are communities consisting of photosynthetic (i.e., cyanobacteria, eukaryotic algae, lichens, and bryophytes) and non-photosynthetic organisms, such as heterotrophic bacteria and microfungi (Chamizo et al., 2013), covering around 12% of the Earth's terrestrial surface (Rodriguez-Caballero et al., 2018). As light dependent associations, they colonize the topsoil layer, where they aggregate soil particles via organic exudates and filamentous structures (Belnap and Büdel, 2016). While biocrusts cover a wide range of latitudes, from tropical to temperate and polar ecosystems (Belnap et al., 2001), they are particularly abundant across arid and semiarid regions, where vascular plants are not able to outcompete them (Belnap and Lange, 2013). Across suitable habitats, they can cover up to 100% of the terrestrial surface throughout the year (Kleiner and Harper, 1972).

Biocrusts have been identified as a key ecological component across many arid and semi-arid regions regarding biodiversity, ecosystem functions.They promote both primary and secondary succession and drive many ecological processes associated to early communities, such as biogenic weathering, soil development, nutrient uptake, and water balance (Belnap, 2001a; Chamizo et al., 2016). In bare soil spots, biocrusts follow a successional process initiated by cyanobacteria and algae, which favor the subsequent establishment of lichens and bryophytes (Belnap, 1995, 2001a). In this perspective biocrusts can facilitate, inhibit or have neutral effects on succession in vascular plant communities (Zhang et al., 2016), thus acting as ecosystem engineers. Biocrusts have been proposed to affect nutrient availability for vascular plants by fixing considerable amounts of nitrogen and carbon, enhancing phosphorous availability and reducing nutrient leaching through soil profiles (Elbert et al., 2012; Barger et al., 2016; Sancho et al., 2016). Due to their carbon and nitrogen fixing abilities, biocrusts have been found to be major players of the global nitrogen cycle and also affect the global carbon cycle (Elbert et al., 2012). Moreover, biocrusts improve soil aggregation, stability and porosity (Belnap, 2006; Castillo-Monroy et al., 2011) largely influencing soil attributes, such as moisture, hydrology and susceptibility to erosion (Bu et al., 2015; Belnap and Büdel, 2016). In addition to soil conditions and nutrient budgets, these organisms can directly affect seed germination of vascular plants, either positively or negatively, indicating that biocrust-mediated processes have relevance beyond simple soil development (Deines et al., 2007; Song et al., 2017).

Overall, biocrusts are composed of mainly slow-growing organisms highly sensitive to their close environment, including soil attributes, microclimatic conditions, and vascular plant communities. In addition to a potential competition for water, nutrients and light, vascular plants provide shaded habitats, and cause litter deposition on biocrusts, what has been considered a negative effect on biocrust colonization, performance and development toward late successional stages (Boeken and Orenstein, 2001; Berkeley et al., 2005). Biocrusts are also sensitive to human disturbances such as trampling by livestock (Condon and Pyke, 2018), soil degradation (Belnap and Gillette, 1998) and invasion by vascular plants (Belnap et al., 2006). Disturbed biocrusts usually experience changes in their physiological performance, taxonomic and ecological composition (Concostrina-Zubiri et al., 2014; Mallen-Cooper et al., 2018), which often represents a retrogressive succession from late (abundant lichens and bryophytes) to early successional stages (only cyanobacteria). As biocrust-mediated processes and services depend on the successional stage, human impacts can deplete them (Belnap, 1995, 2006). To provide an example, trampling by livestock can deteriorate biocrusts, causing soil degradation and a cascade of effects such as increased wind erodibility (Belnap et al., 2007), loss of key ecosystem processes like water infiltration (Chamizo et al., 2016), or favoring exotic plant invasion (Eldridge et al., 2010). In synthesis a myriad of interconnected variables drive biocrust spatial organization, performance, diversity, successional dynamics, and related services and impacts.

Research about the presence of biocrusts, their taxonomic composition and functional role in different habitats as well as their susceptibility to disturbance have initially been concentrated in arid and semiarid regions (Belnap et al., 2001). In most situations, there is a clear climatic limitation to vascular plants, implying that the relationships between biocrust and vascular plants can't be properly assigned as well as the role played by biocrusts on successional trajectories or vegetation dynamics beyond biocrust succession itself (Duane Allen, 2010). In fact, little attention has been devoted to tropical ecosystem dominated by vascular plants, such as dry forests, where according to theory and the state of research, biocrusts are not expected to be either abundant or ecologically relevant (Belnap et al., 2001; Maestre and Cortina, 2002; Seitz et al., 2017). To the best of our knowledge, there are only two studies on biocrusts in dry tropical forests (Maya and López-Cortés, 2002; Büdel et al., 2009) and apparently South America has been entirely overlooked in the context of biocrust research (Büdel et al., 2016). This scientific negligence may be partly due to the relative recency of global change phenomena. In fact, most of the tropical

forest landscape is moving toward human-modified landscapes, usually represented by the establishment of land-use mosaics including active and old crop fields, pasture lands and patches of native forests (Tabarelli et al., 2010). This is particularly true in the Caatinga dry forest of northeast Brazil, the largest and one of the most species-rich seasonally dry tropical forests worldwide (Silva et al., 2017). Slash-and-burn agriculture and free-ranging goat/cattle has transformed the old-growth forest into a temporally dynamic mosaic consisting of old fields (i.e., abandoned crop or pasture fields), regenerating forest patches of varying ages and remaining old-growth forest patches at the landscape spatial scale (Tabarelli et al., 2017). This dynamic mosaic probably offers at least temporal windows of opportunity for biocrusts (i.e., open or sun-exposed habitats) with their spatial and ecological organization and potential impacts and services largely mediated by livestock and land use dynamics.

The effects of biocrust diversity and structure on dry tropical forests remains unstudied, especially with respect to ecosystem service provision, as for example carbon sequestration and soil fertilization. Soil organic carbon (SOC) accumulation can be considered an especially important ecosystem service for the naturally poor soils of the Caatinga (Tiessen et al., 1998). SOC is connected to numerous ecosystem functions upon which humans depend in the Caatinga, so that changes will have immediate consequences for the local population (Thomas, 2012). As in other drylands, SOC in the Caatinga is concentrated in the uppermost centimeters (Schulz et al., 2016) and therefore very sensitive to disturbance (Althoff et al., 2018). As this corresponds with the biocrust stratum, their presence might have a great impact. In drylands and areas at the stage of primary succession, such as in the Caatinga after land abandonment, SOC sequestration is mediated mainly by biocrusts (Lange, 2001; Thomas et al., 2011; Thomas, 2012). Possible positive effects of soil fertilization by biocrusts could be lost in the future, as the Caatinga is highly threatened by climate change and land use and land-use-driven reductions in SOC stocks have been reported for biocrusts (Thomas et al., 2011). The Caatinga is poorly understood in terms of biogeochemical cycling in general (Moura et al., 2016; Althoff et al., 2018) but more so for biocrust influence. Nonetheless, because of its size it may play an important role in global nutrient cycles and could act as a potential sink for atmospheric CO2.

The climate of dry forests in general should offer a suitable habitat for biocrusts (Rodriguez-Caballero et al., 2018), but have never been investigated for their presence or ecosystem services there. As has been known from studies worldwide, biocrusts can provide essential ecosystem services and are responsible for ecosystem integrity in many different habitats. Therefore, we aimed to investigate if biocrusts are an abundant component of dry forests and if they provide ecosystem services essential to the survival of a dry forest under human pressure, using the Caatinga as an exemplary forest biome. Here we present a baseline study on the occurrence, diversity, and ecological role of biocrusts inhabiting a human-modified landscape of the Caatinga dry forest in northeast Brazil. Biocrusts and drivers of biocrust organization at landscape scale were systematically recorded across a network of 34 permanent plots covering a wide range of land use from regenerating forest stands following slash-and-burn agriculture to old-growth forest patches. We expected that (1) biocrust cover, (2) taxonomic composition and (3) successional stage of biocrusts will be affected by forest successional stage (regenerating vs. old growth), litter cover (as a proxy of competition with vascular plants for light), precipitation patterns, goat trails/feces, and soil compaction Additionally, we investigate the impact of human disturbance and variable annual rainfall patterns on the ecosystem service of carbon sequestration and biocrust growth. We expected that human disturbance would severely limit the ability of biocrusts to capture/ store carbon by changing the species composition of the biocrust and their growth- We also expected that the reduction of SOC in biocrusts, induced by multiple low rain years could be canceled out by a single strong precipitation year. We highlight the unexpected diversity supported by biocrusts and the complex relationships between biocrusts and human disturbances, particularly how biocrusts can benefit from, but also potentially affect forest resistance and resilience across human-modified landscapes.

MATERIALS AND METHODS

Study Area

This study was carried out in the Catimbau National Park, a human-modified landscape of the Caatinga dry forest in northeast Brazil, with a predominance of Cactacea, Euphorbiaceae, and Fabaceae (Rito et al., 2017). The 607-km^2 landscape consists of a vegetation mosaic, resulting from the presence of small farmers devoted to slash-and-burn agriculture and livestock grazing (Tabarelli et al., 2017). Active and abandoned crop fields, second-growth forest patches of varying ages, and old-growth forests prevail. All vegetation types are exposed to chronic anthropogenic disturbance through firewood and forage collection, timber exploitation and livestock browsing (Arnan et al., 2018; Souza et al., 2019). Canopy cover never achieves 80–90% (M. Tabarelli personal information and **Figure 1A**). Soil is composed of sedimentary, deep lithosols with quartz sands, with the presence of sandstone outcrops; soils are naturally unfertile with pH around 4.5 (SNE, 2002). The regional climate is semi-arid (<0.65 precipitation/ potential evapotranspiration) with an annual temperature of 23°C (Sampaio, 1995). In Catimbau, annual rainfall varies between 480 and 1,100 mm across the landscape, with rain concentrated between March and July (water deficit between August and February; SNE, 2002) and high spatial and temporal variations, including droughts lasting more than a year (Sampaio, 1995; Rito et al., 2017).

Human Disturbance and Biocrust Spatial Organization

To investigate the spatial distribution and organization of biocrusts at the landscape level we adopted a sampling design based on 34 × 0.1 m^2-permanent plots as follows: 19 old-growth forest plots and 15 secondary growth forest stands with stands ranging from 5 to 70 years after land abandonment; i.e., regenerating stands of varying ages and vegetation structure from almost bare-soil plots up to well-developed forest achieving over

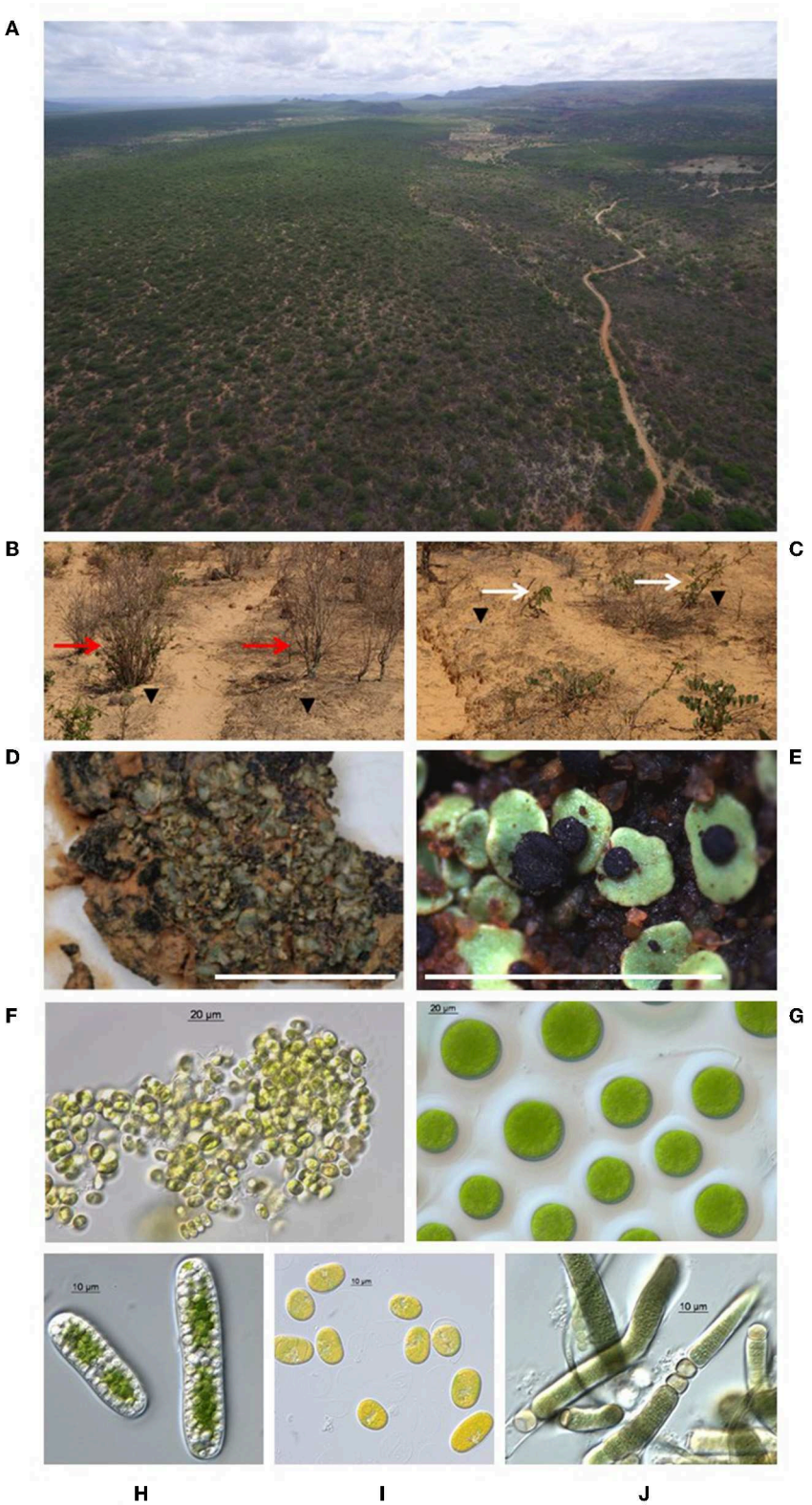

FIGURE 1 | Biocrusts of the Caatinga dry forest in the Catimbau National Park, northeastern Brazil. Overview of the canopy openness in old-growth forests with agricultural farmlands in the background (photo courtesy of Jens Brauneck, University of Kaiserslautern) **(A)**. Trampling paths of goats left in the otherwise closed

(Continued)

FIGURE 1 | biocrust cover **(B)** as well as association of biocrusts with vascular plants **(C)**; shrubs marked with a red arrow *Croton argyrophylloides* **(B)** and shrubs marked with a white arrow *Jatropha mutabilis* **(C)**. In both images the crusts are dominated by dark cyanobacteria (*Nostoc spp., Scytonema sp.* and *Microcoleus vaginatus*) demarcated with black triangles. Light microscopic images of cyanobacteria and green algae isolates **(F–J)**, as well as lichen species [*in situ*, **(D,E)**; white bar represents 500 μm], new to biocrusts of South America and found in Catimbau National Park. *Heppia conchiloba* **(D)**, *Bibbya cf. albomarginata* **(E)**, *Scenedesmus* sp. **(F)**, *Follicularia* sp. **(G)**, *Cylindrocystis brebissonii* **(H)**, *Scotelliopsis cf. rubescens* **(I)**, *Macrochaete lichenoides* **(J)**.

120 Mg ha^{-1} of aboveground biomass (Souza et al., 2019). Plots also covered a gradient of precipitation (510–940 mm of annual rainfall) and of chronic human disturbance, particularly firewood collection and livestock browsing as detailed by Arnan et al. (2018) and Rito et al. (2017). We thus considered to have included a considerable habitat variation resulting from the old-growth forest encroachment by small farmers; i.e., a typical Caatinga human-modified landscape (Tabarelli et al., 2017). The 34 plots were sampled three times during a 12-mo period (August 2017 to July 2018), with plots being regularly sampled every 4 months.

Plots were sampled by adopting a square grid sizing 0.5 × 0.5 m (0.25 m²) made of PVC tubes, subdivided with string into twenty-five cells of 0.1 × 0.1 m (0.1 m²). The grid was disposed on the ground every 10 m, along two 50 m-transects, summing up 10 grids per plot. Each plot was sampled three times during 1 year, with transects disposed at different spots inside the plot every sampling period. It resulted in a total of 1,020 grids; i.e., 34 plots sampled via 10 grids each of the three sampling periods. As grid-based variables we adopted: (1) biocrust cover, (2) cover by herbs and seedlings up to 50 cm tall, (3) ground litter biomass (kg ha-1), (4) goat dung pellets, (5) goat trail cover, and (6) soil penetrability. Biocrust cover was quantified using the point intercept method (Levy and Madden, 1933) adopting three succession-related categories: (a) cyanobacterial biocrust, (b) lichen biocrust, and (c) bryophyte biocrust. Biocrust cover divided into functional groups has long been implemented as a basic variable in biocrust ecology research (Eldridge and Rosentreter, 1999). The point-intercept method was also adopted to estimate plant and goat trail cover. Soil compaction was measured by a penetrometer produced with a sharp steel bar 1.5 m tall, which was thrown into a 32 mm PVC tube (1.5 m long) against the ground (Passos and Oliveira, 2004). Vascular plant cover, trampling by livestock and soil texture/consistency have been identified as key drivers of biocrust performance and occurrence (Belnap and Gillette, 1998; Condon and Pyke, 2018). In this context, forest successional stage (old-growth vs. regenerating stands), cover by herbs, and the amount of ground litter were considered as proxies of total vascular plant cover, while dung and trails by goats as proxy of disturbance. Additionally, mean annual precipitation amount per plot, calculated by WordClim data, was investigated, because water availability is an important driver for biocrust occurrence and succession (Belnap et al., 2006). To investigate the effect of successional stage of the vegetation on biocrust distribution further, for each plot frequency of biocrust occurrence was calculated based on all 30 grids per plot. The available environment for the occurrence of biocrusts presented great physical and biological heterogeneity. Across the 34 plots, ground litter ranged from 29.1 kg ha-1 to 524.7 kg ha^{-1} (195.4 ± 120.3), the coverage by goat trails between 0 and 88.3% (39, 6 ± 21.5), soil compaction (penetrability) between 3.8 and 17.7 cm (10.7 ± 2.8), the amount of feces between 0.0 and 48 pellets/m²(16 ± 14.4), herb cover between 0.0 and 54.5% (8.7 ± 12.1), and precipitation ranging from 510 to 940 mm (748 ± 145).

Finally, biocrust cover was also estimated via point-intercept grids into a network of long-standing goat trails crossing a degraded pasture field established after clearing a large patch of old-growth forest. This pasture field supported immense biocrust patches of *Nostoc* spp., *Scytonema* sp. and *Microcoleus vaginatus* (pers. obs. BB and MS), which were closely associated to small shrubs such as *Croton argyrophylloides* and *Jatropha mutabilis*. (Euphorbiaceae) (**Figures 1B,C**). We randomly selected 30 sampling points along a total of 100 m of goat trails (i.e., trail grids), at least 10 m apart from each other. Paired control grids were placed next to the trail grids (5 m at maximum) but clearly apart from goat trail influences. Point intercept measurements were carried out once in the beginning of the 2017-dry season.

Taxonomic Identification and Microscopy

In order to obtain a basic and rather qualitative understanding of the natural species richness of biocrusts occurring in the Catimbau landscape, including taxonomic and ecological groups, two non-systematic surveys were carried out, outside the 34 0.1 ha plots. First, biocrusts were actively searched in 10 sites, covering a wide range of habitat types representing different successional stages and degrees of disturbance, from abandoned crop and pastures to old-growth forest patches. The sites were chosen as distinctly differing successional stages and under different disturbance regimes, to represent the mosaic-like structure of the Caatinga vegetation. A basic understanding of biocrust presence in the Caatinga was gained and found species were taxonomically assigned. Samples were collected haphazardly by pressing inverted petri dishes (ø 10 cm) into the biocrust and carefully detaching it from the soil matrix with the aid of a spatula. At the field site, biocrusts were identified to the lowest taxon possible using a 10 x magnifying glass, and a light microscope (400 x). Second, biocrusts of two study sites representing the extremes of the disturbance gradient (see soil organic carbon measurements) were identified, using a culturing approach following (Jung et al., 2018) for three samples at both sites. For each sample, 250 mg of biocrust material was randomly picked from a natural sample (one petri dish) and incubated in 15 ml liquid Bold's Basal Medium with soil extract (BBM; Bischoff, 1963) overnight. The samples were being shaken and allowed to settle for 30 s, to remove floating particles. The supernatant was then added to 15 ml BBM. This was repeated three times, resulting in 45 ml sample in BBM medium. After centrifugation for 5 min at 1,000 rpm, the supernatant was decanted and the pellet resuspended in 250 ml of double-distilled water, from which the samples were transferred to solidified

BBM with soil extract, with two replicates for each sample. The cultures were kept in a culture cabinet at standard conditions used in our lab (15–17°C, light-dark cycle of 16:8 h, light intensity of ca. 20–50 μmol photons m^{-2} s^{-1}). The cyanobacterial and green algal colonies were examined with a light microscope (Axioskop, Carl Zeiss, Jena, Germany, 630 x) operated with Zeiss Axiovision software after 5, 12, and 17 weeks. The organisms from the cultures and field samples (also including bryophytes and lichens, were determined with taxonomic keys (e.g., Ettl and Gärtner, 1996; Komárek and Anagnostidis, 1998, 2005), as well as own (B. Büdel) and external expert knowledge (K.C. Pôrto, UFPE).

Soil Organic Carbon Sequestration and Biomass Increase

For this investigation, we chose two case study sites representing contrasting successional stages of biocrust communities along the disturbance gradient of the Catimbau National Park (referred to henceforth as "Early site" and "Late site"). While the Early site is an actively managed Cashew plantation (3.96 ha in size), the Late site (2.64 ha) represents former agricultural land, mainly pasture, on which a young secondary forest with shrub vegetation developed following abandonment ca. 40 years ago (details see **Table S1**). Both sites were investigated for biomass increase and carbon sequestration before the rainy seasons of the years 2017 and 2018. Since the biomass increment of biocrusts of a given year should primarily reflect growth conditions of the previous years, the status of the 2017 biocrust represents 2016, which was a drought year, while 2018 represents 2017, which was an unusually wet year (see **Table S2**). Biocrust coverage was studied in March 2017 only.

To explore the impact of biocrusts on SOC and assess their biomass contribution, bare soil ($n = 16$) and biocrust ($n = 45$) samples were collected from random biocrust and control patches of differing size (biocrusts: 0.0035–1.2462 m^2; control: 0.0067–0.5519 m^2), in both study sites for both years. Distance between the patches and between the controls was always >5 m. For each patch a cylinder of 1 cm depth, which is adequate for biocrusts (Maestre et al., 2013), and 2.5 cm diameter was pushed into the biocrust 7 times and thereafter mixed, to extract enough material for the analyses. The biocrust samples were covered 100% by cyanobacterial biocrusts in both sites; bryophyte and/or lichen presence was avoided to ensure sample homogeneity. Cyanobacterial biocrusts are of key interest, as they are the most dominant group at both sites and throughout the whole national park (**Figure 2B**). In the laboratory, the samples were separated into two fractions, one for quantifying biomass and growth using the amount of chlorophyll *a* as biomass proxy for autotrophic biocrusts (e.g., Johnson et al., 2012), the other for soil organic carbon (SOC) measurements.

To assess biocrust biomass and, hence, growth between 2017 and 2018 for both case study sites, chlorophyll *a* was extracted with dimethyl sulfoxide (DMSO; Ronen and Galun, 1984) and determined spectrophotometrically. For this, each sample was mixed with a scoop tip of $CaCO_3$, covered in DMSO, and heated in a water bath at 65°C for 45 min twice. After centrifugation at 2000 rcf for 10 min, the optical density (OD) of each sample was measured with a spectrophotometer (UV/Vis Spectrometer LAMBDA 35, Perking Elmer Inc. Waltham, MA, USA) at two wavelengths: 700 and 665 nm. Chlorophyll content was then calculated for each sample with the following equation (Arnon, 1949): Chlorophyll *a* (μg) = [(OD_{665} * OD_{700}) * 12.19] * mL DMSO, where OD_{700} is the unspecific absorption and OD_{665} the absorption peak for chlorophyll *a*.

SOC content was determined by the loss on ignition method (LOI) described by Black (1965). Samples were prepared following the protocol of Throop et al. (2012), by sieving them through a 2 mm sieve to exclude stones, passing other aggregates through the sieve, and discarding the skeletal fraction. About 10 g were ground finely in an oscillating mill for subsequent ignition at 550°C for 2 h. SOC stock was calculated according to Schulz et al. (2016) to provide results comparable to those of other surveys: SOC stock [g cm^{-2}] = bulk density [g cm^{-3}] * SOC content [g kg^{-1}] * depth [cm].

Data Analysis

For the assessment of biocrust cover in 34 plots across the park landscape, the point-intercept data from three sampling campaigns were collapsed into a single sample per plot (i.e., 30 grids per plot). Accordingly, the number of recorded cells containing biocrusts, goat trails or vascular plants was transformed into a percentage of the total cells evaluated in the plot (i.e., 30 grids vs. 25 cells per grid per plot). The data were successfully tested for normal gaussian distribution and homogeneity of variance prior to statistical analyses. Pearson's correlation coefficient was used to test potential relationships among explanatory variables and because of that we finally adopted: goat trail cover (%), fine litter biomass (kg ha^{-1}), soil compaction (penetrability in cm) and precipitation gradient (mm). Generalized linear mixed models (GLMM) were performed to examine potential effects of these variables plus forest successional stage (old-growth vs. regenerating stands) on cover of: (1) total biocrust, (2) cyanobacterial crust, (3) lichen-, and (4) bryophyte-dominated biocrust, with plot as the random factor and LME as the estimation method. All analyzes were performed in the R 3.0.0 programming language environment using the packages nlme, stats, mlmRev, lme4, gplots, psych and Rcmdr. Additionally, the difference in biocrust frequency between the two vegetation successional stages was investigated with a global χ^2 test, followed by a pairwise *post hoc* test. The *p*-value was adjusted by Bonferoni correction. The analysis was performed using Statistica (Statistica, version 10, StatSoft, Inc., Palo Alto, CA, USA).

For the differences in biocrust-related biomass increase and SOC sequestration at the two case study sites, a multi-factorial ANOVA was used, with site (succession), study year (precipitation) and bare soil vs. biocrust (biocrust effect) as explanatory variables. The data were normally distributed and homogenous in variance. The analysis was also performed using Statistica.

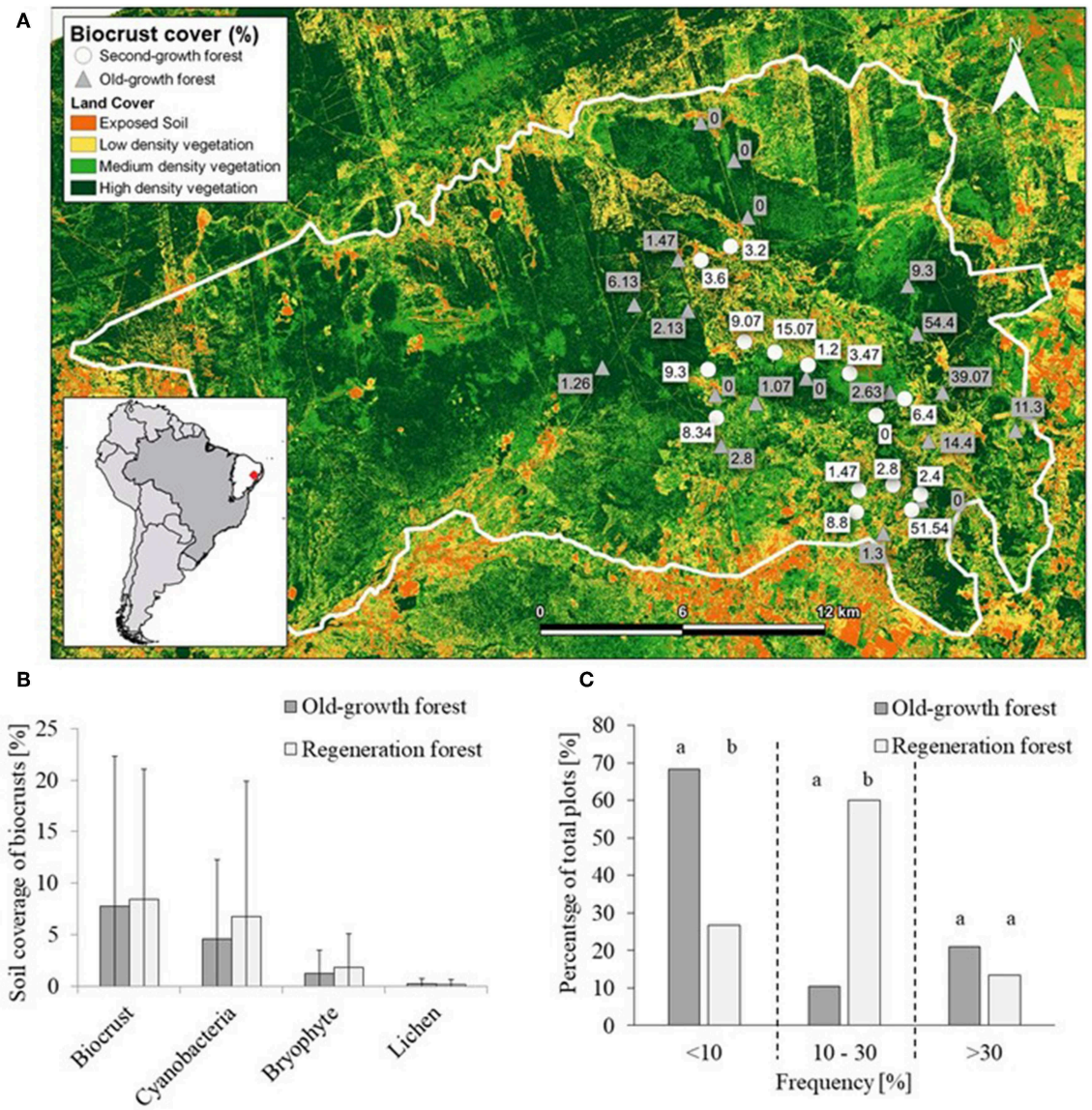

FIGURE 2 | Coverage and frequency of biocrust communities in the Catimbau National Park, northeastern Brazil. Map representing vegetation density (generated with rapideye bands reflectance by Davi Jamelli, Federal University of Pernambuco) and average cover of biocrusts inside the 34 permanent plots **(A)**. Difference in mean biocrust coverage (± SD) between two successional stages of vegetation (old-growth forest $n = 19$; regeneration forest $n = 15$), separated into functional groups of biocrusts **(B)**. Proportion of plots with low (<10%), intermediate (10–30%) and high (>30%) frequency of biocrust occurrence in the two forest successional stages **(C)**. Frequencies generally differed between old-growth and regeneration forest (global $\chi^2 = 9.55$, df = 2, $p = 0.008$); for pairwise test results see text. Significant differences between old growth and second growth forest are denoted with different letters above the bars following a pair wise chi-square *post hoc* test ($p < 0.05$).

RESULTS

In total, our biocrust surveys including the active search across 10 sites, random sampling in all 34 permanent plots, and the culturing approach using samples from the two case study sites, resulted in 50 biocrust taxa throughout Catimbau National Park. More precisely, 19 biocrust organisms were identified to the species level, 33 to the genus, and one taxon to the family. A total of 23 taxa have not been reported yet for South America, and hence the Caatinga in the context of biocrust research (**Table 1** and **Figures 2D–J**). They include cyanobacteria (three taxa), green algae (nine taxa), lichens (seven taxa) and bryophytes (four taxa). The cyanobacteria *Nostoc* spp., *Scytonema* sp., the liverworts *Riccia* sp. 1 and sp. 2, and the moss *Bryum exile* were the most frequent taxa. While the number of cyanobacteria, bryophytes and green algae species was similar under contrasting rainfall and disturbance intensities, more lichen species occurred in wetter areas (**Table 1**).

At the plot spatial scale ($n = 34$), biocrusts of all three successional stages were recorded throughout the year, although in six plots (17.6%), no biocrust was recorded during the three sampling campaigns (**Figure 2A**). However, most of the plots

TABLE 1 | Biocrust taxa found in the National Park Catimbau, northeastern Brazil, roughly assigned to their occurrence in high and low precipitation and disturbance regimes.

Species	High rainfall (>748 mm)	Low rainfall (≤748 mm)	High disturbance	Low disturbance
Cyanobacteria				
Aphanocapsa sp.	■		■	
Calothrix sp.		■		■
Chroococcidiopsis sp.		■		■
Gloeocapsa sp.	■		■	
Leptolyngbya sp.	■		■	
Macrochaete lichenoides	■		■	
Microcoleus vaginatus	■	■	■	■
Nostoc edaphicum	■		■	
Nostoc ellipsoides	■		■	
Nostoc sp. 1	■	■	■	
Nostoc sp. *2*	■	■	■	
Oscillatoria sp.	■	■	■	
Pseudophormidium sp.		■		■
Schizothrix sp.	■	■	■	■
Scytonema hyalinum	■		■	
Scytonema sp. 1	■	■	■	■
Stigonema sp.		■		■
Tolypothrix sp.		■		■
Green algae				
Chlorella sp.		■		■
Cylindrocystis brebissonii	■		■	
Desmococcus sp.	■		■	
Follicularia sp.	■		■	
Heterococcus sp.	■	■	■	■
Klebsormidium sp.	■		■	
Macrochloris multinucleata		■		■
Neochloris sp.	■	■	■	■
Scenedesmus sp.		■		■
Scotiellopsis rubescens	■	■	■	■
Spongiochloris sp.	■		■	
Stichococcus sp.				
Trebouxiophyceae		■		■
Green algae sp. 1	■	■	■	■
Green algae sp. 2	■		■	
Green algae sp. 3	■		■	
Green algae sp. 4	■	■	■	■
Mosses				
Bryum argenteum		■		■
Bryum exile	■	■	■	■
Campylopus pilifer	■		■	
Fissidens submarginata	■		■	
Tortela humilis	■	■	■	
Liverworts				
Riccia sp. 1	■	■	■	
Riccia sp. 2	■	■	■	
Riccia vtialii		■		■

(Continued)

TABLE 1 | Continued

Species	High rainfall (>748 mm)	Low rainfall (≤748 mm)	High disturbance	Low disturbance
Lichens				
Buellia sp.	■			
Cladonia foliacea	■		■	■
Cladonia verticillaris	■		■	
Heppia conchiloba.		■		■
Lecidea sp.	■			■
Peltula michoacanensis		■		■
Bibbya cf. albomarginata	■		■	■

Precipitation is categorized as high if the plot a species has occurred in has a higher mean annual rainfall than the mean annual rainfall across all investigated plots (748 mm). Disturbance is categorized according to Arnan et al. (2018), where plots with chronic anthropogenic disturbance values (GMDI) higher than 34 (mean of all plots) were considered highly disturbed, and below or equal to 34 as low disturbed sites. Morphospecies were assigned as sp. 1 – 4 through comparison of the cell sizes of 50 cells and 20 heterocytes, if present in the species. Species that have not yet been registered for biocrusts in South America are marked in red. Gray color indicates that the species has been found in the respective category of disturbance or precipitation regime.

(44.4% of 27 plots with biocrusts recorded) supported only cyanobacteria biocrusts (i.e., initial biocrusts), particularly those dominated by *Scytonema* sp. Only 7.4% of plots exhibited lichen biocrusts, particularly *Bibbya* cf. *albomarginata* (intermediate successional biocrusts), while 48.2% had bryophytes recorded; i.e., late biocrusts with mosses and liverworts. Total plot cover of biocrusts ranged from 0 to 54.4% (8.1 ± 13.6; mean ± standard deviation) with no difference between successional stages of the vegetation [$t_{(34)} = -0.146$, $p = 0.885$] (**Figure 2B**). Frequency of biocrust coverage however, did show a difference between the two successional stages of the forest. Regenerating forest supported more plots with moderate biocrust frequency between 10 and 30% (χ^2 (1; $N = 11$) = 9.38, $p = 0.002$]. On the other hand, more patches of low biocrust frequency (<10%) where found in old-growth forest [χ^2 (1; $N = 17$) = 5.85, $p = 0.016$] (**Figure 2C**). Plots with high crust frequency (>30%) were seldomly and evenly encountered across forest successional types [χ^2 (1; $N = 6$) = 0.34, $p = 0.56$]. Considering only the 27 plots in which biocrusts occurred, cover by cyanobacterial biocrusts (initial) ranged from 0.13 to 53.1% (7.5 ± 11.5), lichen biocrust (intermediate) between 0.26 and 1.8% (1.24 ± 0.6) and bryophyte biocrust (late) between 0.11 and 9.3% (3.8 ± 3.2).

Across plots, biocrust cover decreased with (1) greater coverage of goat trails, (2) higher fine litter biomass, and (3) higher soil penetrability (**Table 2**, **Figures 3A–C**), while other factors such as precipitation, herb cover, or feces showed no effects. The cyanobacteria biocrust responded negatively to litter (**Figures 3D–F**, **Table 2**), while no significant relationship was found between the coverage by lichen biocrusts and the explanatory variables (**Figures 3G–I**, **Table 2**). Bryophyte biocrusts negatively responded only to goat trails (**Figures 3J–L**, **Table 2**), but positively to soil compaction. The successional stage of the vegetation (regenerating vs. old-growth forest) did not directly affect biocrust coverage, but its interaction with the soil compaction affected both the total cover of biocrusts and the cover of bryophytes, this interaction being stronger across old-growth forests (**Table 2**). Finally, long-standing goat trails supported only 6.3 ± 6.1% of biocrust cover, while spots of degraded pastures reached 23.0 ± 2.6% of the soil surface covered by biocrusts [$t_{(30)} = 13.5$, df = 39.50, $p < 0.0001$].

SOC content in bare soil (control) was equal for both case study sites (Tukey *posthoc*: $p_{2017} = 0.58$; $p_{2018} = 0.56$). Therefore, site differences between biocrusts are solely due to the successional status of the biocrust communities themselves [$F_{\text{succession}(1,236)} = 71.1$, $p = 0.000$]. SOC content roughly doubled in biocrusted soils compared to bare ground (from 6.04 and 8.70 g kg^{-1} to 10.67 and 19.82 g kg^{-1}, respectively for Early and Late site) [$F_{\text{biocrust effect}(1,236)} = 145.55$, $p = 0.000$], independent of site and precipitation. Likewise, SOC doubled in late as compared to early successional biocrusts (10.67 to 19.82 g kg^{-1}) (Tukey posthoc: $p_{2017} = 0.000$; $p_{2018} = 0.000$; **Figure 4A**). Precipitation did not show any effect on SOC, neither in biocrusts nor in control soils [$F_{\text{precipitation}(1,236)} = 0.037$, $p = 0.85$, **Figure 4A**].

Likewise, the control soils of both case study sites had similar Chlorophyll a concentration as a proxy of biomass of autotrophic biocrusts (Tukey *posthoc*: $p_{2017} = 1.000$; $p_{2018} = 0.999$), so that differences are a result of biocrust successional status [$F_{\text{succession}(1,236)} = 11.3$, $p = 0.001$]. In the wet year, the presence of biocrusts increased the chlorophyll a content of top soil layers by more than 1,500-fold (from 0.0036 to 5.85 mg m^{-2}) in the early successional crusts and 12-fold (from 1.25 to 15.82 mg m^{-2}) in late successional biocrusts (Tukey *posthoc*: $p_{\text{early}} = 0.04$; $p_{\text{late}} = 0.000$; **Figure 4B**) compared to bare soil. Biocrust presence in general showed a pronounced increase of chlorophyll a content in the soil [$F_{\text{biocrust effect}(1,236)} = 37.4$, $p = 0.000$]. Precipitation had a strong effect on biocrust growth across the study years: From the drought year 2017 to the wet year 2018, chlorophyll a increased 7 times at the Early site (from 0.71 to 5.85 mg m^{-2}) and 6.5 times (from 2.12 to 15.85 mg m^{-2}) at the Late site [$F_{\text{precipitation}(1,236)} = 27.9$, $p = 0.000$].

DISCUSSION

Our results suggest that human-modified landscapes of the Caatinga dry forest can support biocrust communities at different

TABLE 2 | Effect of goat trail soil coverage (%, GT), fine litter biomass (kg ha^{-1}), soil compaction (SC, soil penetrability in cm), vegetation succession stage (VSS) and its interaction with soil cover by biological soil crusts (BSC) on richness of BSC morphotypes and cover by cyanobacteria crust, lichen crusts, and bryophyte crusts in the Catimbau National Park, Pernambuco, Northeast Brazil.

Variable	Effect	DF	F	P	R^2_m	R^2_c	Effect	DF	F	P	R^2_m	R^2_c	Effect	DF	F	P	R^2_m	R^2_c
Biocrust coverage (%)	Goat trail	1,30	4.27	**0.04**	0.11	0.89	Litter	1,30	5.84	**0.02**	0.16	0.89	Soil comp.	1,30	6.47	**0.01**	0.34	0.91
	VSS	1,30	0.04	0.84			VSS	1,30	0.04	0.84			VSS	1,30	0.18	0.66		
	VSS*GT	1,30	0.11	0.74			VSS*Litter	1,30	0.66	0.41			VSS *SC	1,30	10.3	**0.003**		
Richness of biocrust morphotypes	Goat trail	1,30	2.28	0.14	0.14	0.89	Litter	1,30	2.95	0.09	0.14	0.89	Soil comp.	1,30	8.26	**0.007**	0.24	0.9
	SSV	1,30	0.70	0.40			VSS	1,30	1.24	0.27			VSS	1,30	2.09	0.15		
	VSS*GT	1,30	2.68	0.11			VSS*Litter	1,30	1.47	0.23			VSS *SC	1,30	0.29	0.58		
Cyanobacteria crust coverage (%)	Goat trail	1,30	3.29	0.07	0.11	0.89	Litter	1,30	5.43	**0.02**	0.15	0.89	Soil comp.	1,30	1.55	0.22	0.12	0.89
	VSS	1,30	0.09	0.76			VSS	1,30	0.44	0.50			VSS	1,30	0.54	0.46		
	VSS*GT	1,30	0.88	0.35			VSS*Litter	1,30	0.01	0.89			VSS *SC	1,30	2.43	0.12		
Lichen crust coverage (%)	Goat trail	1,30	0.31	0.58	0.02	0.88	Litter	1,30	0.20	0.65	0.00	0.87	Soil comp.	1,30	0.78	0.38	0.05	0.88
	VSS	1,30	0.02	0.88			VSS	1,30	0.00	0.95			VSS	1,30	0.00	0.97		
	VSS*GT	1,30	0.87	0.35			VSS*Litter	1,30	0.01	0.92			VSS *SC	1,30	1.29	0.26		
Bryophyte crust coverage (%)	Goat trail	1,30	6.11	**0.01**	0.19	0.89	Litter	1,30	0.40	0.53	0.02	0.88	Soil comp.	1,30	3.41	**0.05**	0.24	0.90
	VSS	1,30	0.09	0.76			VSS	1,30	0.50	0.50			VSS	1,30	0.88	0.35		
	VSS*GT	1,30	0.33	0.56			VSS*Litter	1,30	0.75	0.75			VSS *SC	1,30	5.89	**0.02**		

Significant effects are in bold ($P < 0.05$); DF, degree of freedom; F, effect value; R^2_m, squared R marginal; R^2_c, squared R conditional.

successional stages, with high taxonomic and ecological diversity. Biocrusts composed of at least 50 taxonomic groups occurred across numerous habitats—from abandoned farmlands to old-growth forests, although there was a predominance of initial biocrusts dominated by cyanobacteria. Bryophytes were the second most abundant group, while lichens were almost absent, although being the typically intermediate stage of succession in hot arid/semi-arid areas. Biocrusts covered a significant fraction of the soil surface (8.1 ± 13.64 %; mean ± standard deviation of the biocrusts in the Catimbau National Park) throughout the year, with more than 50% coverage of cyanobacterial biocrusts in some areas. Biocrust communities benefited from reduced ground litter and little goat pressure, while forest successional stage and precipitation amount *per se* had no effect. They were potent carbon sequestering agents of dry forests and due to this fertilization effect could play a substantial role in forest regeneration. However, high levels of anthropogenic disturbance lead to a pronounced decrease in biocrust growth and SOC sequestration.

Biocrust Composition and Diversity

Conventionally, biocrusts are known to predominate in landscapes with little cover by vascular plants (i.e., arid and semi-arid regions dominated by sparse shrubs) and reduced human disturbances (Belnap et al., 2016; Ayuso et al., 2017). Our findings suggest that even in forest biotas, biocrusts can be ecologically/taxonomically diverse and abundant, particularly in the case of rural populations creating vegetation mosaics. Although comparisons across studies are difficult due to differences in sampling effort and geographic scale, the presence of 33 genera only in the Catimbau National Park suggests that the Caatinga supports a relatively diverse community at landscape scale. As an example, in Australia 86 genera were recorded considering 83 sites (Thompson et al., 2006) and more than 60 taxa were found across seven ecosystems in Africa (Büdel et al., 2009). Most taxa found exhibit a broad geographical distribution, occurring with high abundance across deserts and semi-deserts, e.g. *Bryum* (moss; e.g., Kidron et al., 2002), *Riccia* (liverwort; e.g., Eldridge and Tozer, 1996), *Heppia* (lichen, e.g., McCune and Rosentreter, 1995), *Scytonema*, *Microcoleus* and *Nostoc* (cyanobacteria; see Büdel et al., 2016), and *Chlorella* (green algae; e.g., Rosentreter and Belnap, 2001). The list of biocrust species was expanded by 23 taxa that have not yet been registered as biocrust members for the Caatinga, Brazil or South America and included cyanobacteria (e.g., *Macrochaete lichenoides*; **Figure 1J**), green algae (e.g., *Cylindrocystis brebissonii*; **Figure 1H**), lichens (e.g., *Heppia conchiloba*; **Figure 1D**) and mosses (e.g., *Fissidens submarginata*).

The dominant functional group of biocrusts in the study landscape, independent of forest successional stage, was cyanobacteria and, occasionally, green algae, making up 79.3 % of the biocrust-covered area. Cyanobacteria belong to the first colonizers of open soils and throughout the whole succession of a biocrust, are an important component of it (Weber et al., 2016). Both forest systems supporting mainly this biocrust type suggests that the development of later successional stages might be suppressed by irregular rainfall, vascular plant presence or

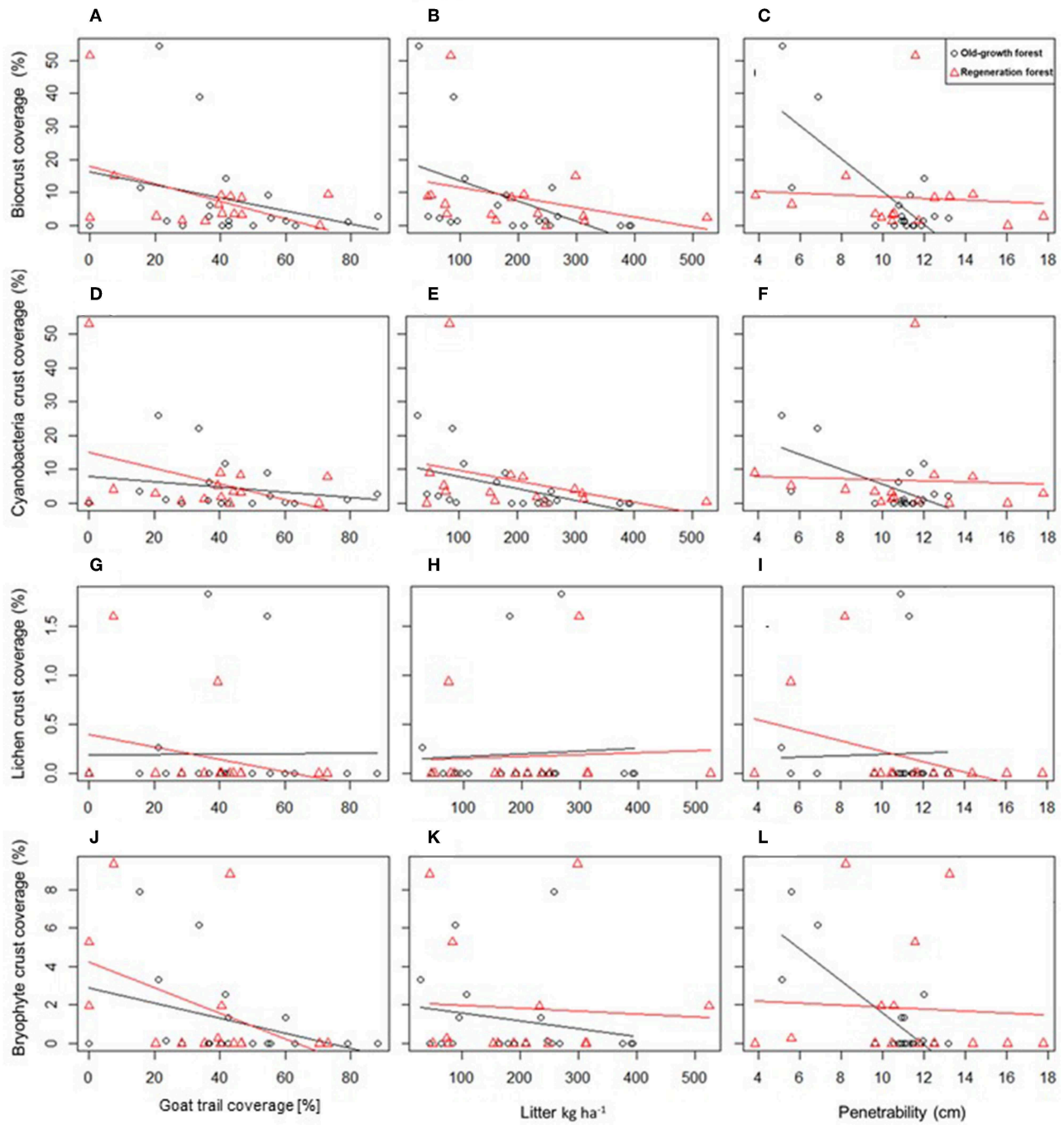

FIGURE 3 | The relationships between biocrust communities of the Caatinga dry forest and goat trail cover (%), accumulated fine litter biomass (kg ha^{-1}) and soil (penetrability cm). Variables and functional groups depicted are: total biocrust cover **(A–C)**, cover of cyanobacteria crusts **(D–F)**, lichen **(G–I),** and bryophyte biocrust **(J–L)** cover across regenerating ($n = 15$) and old-growth forest plots ($n = 19$).

sandy soils. Bryophytes were the second most abundant group while still subdominant (18% of all biocrusts). The almost complete absence of lichen-dominated biocrust communities in every habitat independent of vascular succession in the study landscape (2.7%) contrasts with our current understanding of biocrust succession in drylands (Seppelt et al., 2016; Weber et al., 2016), where lichens, especially of the genus *Collema*, form intermediate biocrust successional stages. The relative lack of lichens in the sedimentary Caatinga may be explained by the facts that they are heavily susceptible to trampling (e.g., Concostrina-Zubiri et al., 2014), dense litter cover (Briggs and Morgan, 2008) and don't grow well on coarse sand (Bowker et al.,

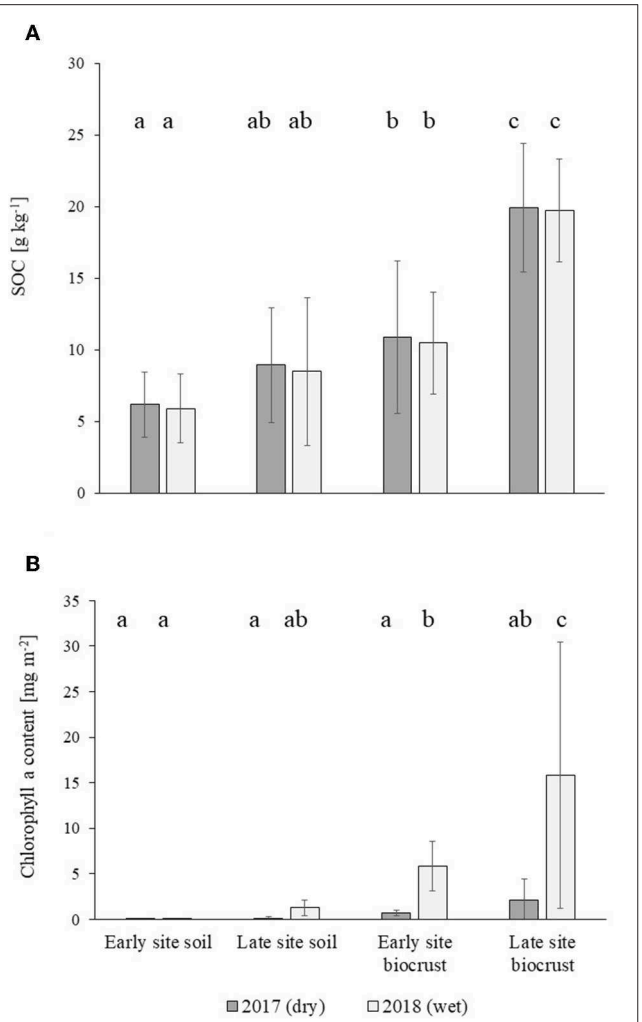

FIGURE 4 | Edaphic characteristics of topsoil layers in bare soils and biocrusts at early and late successional case study sites in the Catimbau National Park, northeastern Brazil. Depicted are means ± SD of soil organic carbon content **(A)** and chlorophyll *a* concentration **(B)** in biocrusts ($n = 45$) and bare soil ($n = 16$). Measurements were done in a year following a drought period (2017) and after a very wet year (2018) (see material and methods for details). Significant differences between means are denoted with different letters above the bars following Tukey's *post hoc* test ($p < 0.05$).

2006). In fact, burial by sand or litter is known to kill lichens, green algae and smaller cyanobacteria (Campbell, 1979) but may promote bryophytes, as some species are able to push through a litter or recent dust layer (Marschall and Proctor, 2004). While we qualitatively observed a tendency for lichen species to occur in wetter areas with lower disturbance intensity, their low overall cover in the study likely compromised statistical power to detect drivers of lichen distribution.

Biocrust Distribution and Anthropogenic Disturbance

Biocrusts in (sub-)tropical forests have either been neglected entirely or described as a transient phenomenon associated to disturbances, such as treefall gaps, that disappear during forest succession (Seitz et al., 2017). This was not the case in the Caatinga, where biocrusts were present at every successional stage and reached similar coverage scores in old-growth and regenerating forests. Most plausibly, the Caatinga dry forest serves as a suitable habitat to biocrusts because light is not as limiting as in rainforests and other forest biomes. Even the old-growth forest permit the occurrence of biocrusts by providing relatively well-illuminated habitats. This is due to its low stature, low leaf area index, and high proportion of deciduousness (Pennington et al., 2009; Oliveira et al., 2013), thus reducing forest biomass and litter production; all attributes associated to a highly seasonal rainfall regime and a semiarid climate; i.e., reduced ecosystem productivity (Eamus, 1999). However, biocrusts benefited from human disturbance as the distribution of biocrusts in regeneration areas was less patchy than in old-growth-forests, where they may primarily occur because of the pronounced discontinuity of the canopy (see **Figure 1A**). As old-growth forests are converted into agricultural-forest mosaics, biocrusts spread over more illuminated habitats, such as old fields (abandoned crops and pastures) and second-growth forest stands of varying ages. Precipitation is usually advantageous for biocrust presence (Bowker et al., 2016) though it comes with the disadvantage of having to compete with vascular plants for light and space (Harper and Belnap, 2001; Thompson et al., 2006). Surprisingly and despite a strong rainfall gradient across the study landscape, precipitation did not show a significant impact on biocrust coverage. This may be explained by (1) the pronounced spatio-temporal patchiness of rainfall in the Caatinga region (Silva et al., 2017), (2) a high wet season rainfall allowing biocrusts to water-saturate and grow (saturating water content for biocrusts see e.g., Szyja et al., 2018), or (3) other factors adding noise to the biocrust distribution pattern. To provide an example, chronic anthropogenic disturbance, e.g., fuel wood collection, represents a continuous factor opening canopies at all regeneration stages, independent of precipitation amount, thus potentially altering biocrust cover and community composition. Similar results have been found by a recent study on the distribution of vascular plant biomass, which could be explained by a multitude of factors, except rainfall (Souza et al., 2019). Moreover, the values used for the analysis of rainfall effects were long-term annual means, thereby ignoring the fact that biocrusts show short-term responses to intensity and frequency of rainfall in individual years. This is illustrated by the very steep increase in biomass following a year of above-average rainfall (**Figure 4**).

In contrast to rainfall, leaf litter is a factor that clearly negatively affected the establishment and growth of total biocrust and cyanobacteria biocrusts in the Caatinga, thus confirming earlier studies (e.g., Boeken and Orenstein, 2001). However, litter did not influence biocrust cover between old growth and regeneration forests, despite differences in standing biomass among stages of forest regeneration (Souza et al., 2019). This may be partly accounted by abundance and density of woody parts of the vegetation, but also be caused by the fact that leaf biomass is known to rapidly reach a plateau as forest stands mature (Tadaki, 1977). To our knowledge, the reported estimate of biocrust cover

in the Catimbau National Park (ca. 8% of the total area), represent the first quantitative assessment for tropical dry forests. Studies in savannas in southern Africa, one of the few investigations done in woodland ecosystems, found biocrust cover reaching <1% (except for one site in Sonop province with up to 20%; Jürgens et al., 2010). It should be emphasized that the Caatinga human-modified landscapes provide suitable conditions for the development of biocrust assemblages that have been traditionally considered "typical" of relatively undisturbed arid habitats (Belnap, 2001b). In the Caatinga, the best environment for biocrust development, considering physiological performance, successional development, ground cover and persistence, consists of habitats free of vascular plants and lack of soil disturbance. But vascular plants can also have a beneficial effect on biocrust presence: Caatinga human-modified landscapes usually support high stock rates; i.e., 4–6 goats per km², particularly across regenerating forest stands and old fields (11 goats per km²; Melo, 2017). Although goats consume litter, intense trampling does not allow biocrusts to settle and develop toward late successional stages, as confirmed by consistent negative relationships between goat trails and biocrusts in this study and previously published studies (Guo et al., 2008; Bowker et al., 2013; Ferrenberg et al., 2017). Goat-induced disturbance, which leads to a lower abundance of biocrust presence, except underneath shrubs, is therefore a plausible mechanism behind the unsuspected positive association observed between biocrusts and small-statured shrubs, when plant presence usually would reduce biocrust distribution. Although more subject to deposition of litter and competition for light, biocrusts beneath small shrubs likely benefit from reduced trampling, particularly beneath less palatable shrubs, such as latex-bearing *Jatropha* and *Croton* species, and reduced evaporation through shading (Bowker et al., 2005; Zhang et al., 2016), especially at sites with high disturbance (Tabeni et al., 2014).

Biocrusts as Ecosystem Service Providers and Their Role for Dry-Forest Resilience

Biocrusts have been reported to influence several ecosystem-level processes, some of which can be considered as ecological services of local and global relevance. Understanding of biogeochemical cycles, including carbon cycling of biocrusts, is limited in the Caatinga (Elbert et al., 2012; Moura et al., 2016; Althoff et al., 2018). Cyanobacteria-dominated biocrusts double the soil organic carbon (SOC) content in the first cm of soil when compared to open soil, at both sites. The higher disturbance at the early site reduced the natural SOC stock in the soil and in the biocrust by half in comparison to the undisturbed site. However, the loss of SOC in bare soil can be neutralized by the presence of biocrusts which nonetheless double the SOC amount in underlying soil. A higher SOC content in the late biocrust is most likely attributed to a different species composition with later successional cyanobacteria (e.g., *Nostoc* sp.) being able to sequester more carbon (Lange, 2001). Interestingly, no difference of SOC content could be detected between either the wet or the dry year, even though one of the dominant organisms in both crusts, *Microcoleus vaginatus*, is highly adapted to rain pulses and should increase the SOC content of the soil during a very rainy season (Ferrenberg et al., 2015; Tucker et al., 2019). This lack of feedback could be explained by the fact that during years with little or no rainfall, both biocrust growth and decomposition is restricted (e.g., Thomas et al., 2011), and net loss in SOC is probably low. Additionally, SOC input might not only be due to increased net primary productivity of the biocrust itself but caused by the dust-trapping mechanism of the rough surface of biocrusts (Belnap, 2003). This is not influenced by the precipitation amount and collects nutrient rich dust, eroded at other sites. This theory is supported by measuring biomass, using chlorophyll *a* as a proxy for net primary productivity, which showed a pronounced increase in biocrust biomass at both sites after the wet year without an increase of SOC. Disturbance had a negative impact on biocrust growth as biomass increase was only one third of that in the undisturbed habitat. The SOC stocks of topsoils with or without biocrusts fit well within the range of reported values for the Caatinga, related to other habitats it is very low (**Table 3**). Values resulting from this study might underestimate the actual SOC input by biocrusts in the Caatinga. Later successional stages including bryophytes and lichens, which have been excluded in this analysis, have higher carbon sequestration values (Lange, 2001) and react different to moisture and temperature changes than cyanobacterial dominated crusts (Tucker et al., 2019). The carbon stored in biocrusts represents ca. 7% of the carbon sequestered in aboveground biomass, estimated in a recent quantification of standing biomass in the Caatinga (Souza et al., 2019). The loss of such a considerable fraction of the overall carbon balance due to climate change or increasing disturbance might seriously threaten this fragile ecosystem and reduce its resilience to mitigate human impacts. While our results suggest a considerable influence of biocrusts on the Caatinga ecosystem, one should be mindful that they reflect carbon sequestration and biocrust growth of two sites at opposite ends of the disturbance gradient. In view of the complexity and patchiness of the human-modified Caatinga (Silva et al., 2017), a future research agenda should therefore aim at exploring additional environmental conditions and stages of forest regeneration. Nevertheless, the control soils of both study sites were equal in SOC and chlorophyll content, indicating that the observed differences were in fact based on biocrust presence and biocrust age. These in turn are shaped by the disturbance regime found at the sites. Filamentous cyanobacteria such as those from the dominating genera in our focal landscapes (i.e., *Scytonema* sp. and *Microcoleus vaginatus*) have been indicated as aggregators of sandy soils (Ferrenberg et al., 2015) thus reducing soil runoff and degradation (i.e., a key service in crop/pasturelands Pimentel et al., 1987). At landscape and regional scale, the Caatinga can be approached as a successional mosaic through which local farmers support livelihood by forest products and services such as recovery of nutrient stocks via forest regeneration. Unfortunately, the Caatinga dry forest has been driven toward desertification over large areas as soils become deeply depauperated due to superficial erosion (i.e., runoff) and nutrient exportation via crops/livestock (Leal et al., 2005; Vieira et al., 2015). Local depletion of forest regeneration sources, such as seeds, seedlings and resprouts can

TABLE 3 | Overview of published and own (highlighted in gray) data on soil organic carbon (SOC) stocks in the Caatinga and other semi-arid ecosystems.

Area/Organism	SOC g kg^{-1}	Depth	Publication
Early Site cyanobacterial biocrust	10.67 ± 4.50	1 cm	This study
Early Site soil	6.04 ± 2.30	1 cm	This study
Late Site cyanobacterial biocrust	19.82 ± 4.04	1 cm	This study
Late Site soil	8.70 ± 4.54	1 cm	This study
Caatinga slash and burn recovering soil	8.61	20 cm	Freitas et al., 2012
Caatinga preserved soil	11.62	20 cm	Freitas et al., 2012
Horqin Desert moss biocrust	6.53 ± 4.63	10 cm	Li et al., 2017
Western Loess Plateau moss biocrust	5.07 ± 0.16	10 cm	Li et al., 2017
Mu Us Desert Cyanobacterial and moss biocrust	10.59 ± 4.93	10 cm	Li et al., 2017
Tengger-Alxa Desert mixed lichen, moss, cyanobacterial biocrust	4.86 ± 0.77	10 cm	Li et al., 2017
Guerbantunggut Desert cyanobacterial and lichen biocrust	2.38 ± 0.62	10 cm	Li et al., 2017
Qaidam Desert cyanobacterial biocrust	0.41 ± 0.17	10 cm	Li et al., 2017
Guerbantunggut desert, moss biocrust *(Bryum argenteum)*	∼ 2.2–5	5 cm	Zhao et al., 2018
Area/Organism	**SOC Mg ha^{-1}**	**Depth**	**Publication**
Early site cyanobacterial biocrust	1.40 ± 0.60	1 cm	This study
Early Site soil	0.77 ± 0.30	1 cm	This study
Late Site cyanobacterial biocrust	2.49 ± 0.54	1 cm	This study
Late Site soil	1.13 ± 0.60	1 cm	This study
Caatinga soil	20.00	10 cm	Tiessen et al., 1998
Caatinga soil	26.20	10 cm	Kauffman et al., 1993
Caatinga soil	4.14	5 cm	Schulz et al., 2016
Caatinga soil	17.00	5–60 cm	Schulz et al., 2016
Caatinga soil	35.13–46.46	40 cm	Barros et al., 2015
Caatinga slash and burn recovering soil	23.15	20 cm	Althoff et al., 2018
Caatinga preserved soil	31.85	20 cm	Althoff et al., 2018
Deserts and Semi Deserts soil	57.00	Across all horizons	Prentice et al., 2001
Tropical Savannas and Grasslands soil	90.00	Across all horizons	Prentice et al., 2001
Tropical Forest soil	122.00	Across all horizons	Prentice et al., 2001
Temperate Forest soil	147.00	Across all horizons	Prentice et al., 2001
Boreal Forest soil	274.00	Across all horizons	Prentice et al., 2001

also be important (Tabarelli et al., 2017). Increased aridity (as predicted by climate change models; Torres et al., 2017) can magnify this human-induced degradation. In this scenario, it is reasonable to propose an unlimited number of connections between biocrusts, soil attributes/amelioration, forest recovery and human well-being/sustainability. As a working hypothesis, soil engineering by biocrusts, for example, enhances crop production, retard soil degradation on crops fields, favor forest recovery after land abandonment and old-growth forest productivity (i.e., higher forest resilience). Facilitation/nucleation promoted by sparse shrub-biocrust association may represent the best opportunity for forest regeneration across degraded habitats covering naturally poor soils, such as those in our focal landscape. In other words, biocrusts are connected to ecosystem resistance/resilience.

In summary, biocrusts seem to be a conspicuous and highly diverse component of Caatinga human-modified landscapes, extending the ecological role played by these associations to a prior overlooked ecosystem type. Furthermore, biocrusts benefit from the establishment of second-growth forests, in which they proliferate, reaching considerable coverage, although they are exposed to the controlling effects imposed by goats, litter cover and soil attributes. Such a positive synergism between human populations and biocrusts may result in a more crucial ecological role played by biocrusts as humans proceed with the encroachment of tropical landscapes, including those covered by forest vegetation. Although the presence of woody vegetation may impose some negative impacts on biocrusts (i.e., competition and litter cover) it also appears to provide protection against intense goat trampling. In this perspective, there must be an optimum combination for biocrusts considering vascular plant cover and goat pressure. Biocrusts act as carbon sequestering fertilizers of the Caatinga soils and their cover benefits from human presence. However, anthropogenic disturbance will lead to a considerable decrease in this ecosystem service provided by biocrusts, even if their coverage is not affected. Considering the intense dynamics of land use provoked by shifting cultivation and livestock breeding in the Caatinga, future studies need to investigate functions and services provided by biocrusts, particularly as drivers for forest regeneration and

as a prevention of further desertification, as they are crucial for sustainable use of the Caatinga.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

RW, MT, IL, BB, MS, and AM conceived and planned the experiment. AM, MS, and FO carried out the experiments with help from BB. MS and AM analyzed the data. BB, RW, and MT contributed to the interpretation of the results. MS took the lead in writing the manuscript. MT, RW, BB, and IL supervised the project. All authors provided critical feedback and helped shape the research, analysis and 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/fevo.2019.00482/full#supplementary-material

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The Compositionally Distinct Cyanobacterial Biocrusts From Brazilian Savanna and Their Environmental Drivers of Community Diversity

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The last decade was marked by efforts to define and identify the main cyanobacterial players in biological crusts around the world. However, not much is known about biocrusts in Brazil's tropical savanna (*cerrado*), despite the existence of environments favorable to their development and ecological relevance. We examined the community composition of cyanobacteria in biocrusts from six sites distributed in the Southeast of the country using high throughput sequencing of 16S rRNA and phylogenetic placement in the wider context of biocrusts from deserts. Sequences ascribable to 22 genera of cyanobacteria were identified. Although a significant proportion of sequences did not match those of known cyanobacteria, several clades of *Leptolyngbya* and *Porphyrosiphon* were found to be the most abundant. We identified significant differences in dominance and overall composition among the *cerrado* sites, much larger than within-site variability. The composition of *cerrado* cyanobacterial communities was distinct from those known in biocrusts from North American deserts. Among several environmental drivers considered, the opposing trend of annual precipitation and mean annual temperature best explained the variability in community composition within Brazilian biocrusts. Their compositional uniqueness speaks of the need for dedicated efforts to study the ecophysiology of tropical savanna biocrust and their roles in ecosystem function for management and preservation.

Keywords: biocrust, *cerrado* savanna, cyanobacteria, *Leptolyngbya*, *Porphyrosiphon*, aridity

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INTRODUCTION

Biological soil crusts (BSCs or biocrusts) are microbial assemblages present at the top soil of several terrestrial ecosystems, especially in drylands. Biocrusts are typically composed of cyanobacteria (Garcia-Pichel et al., 2001) but sometimes also eukaryotic algae, lichens, or mosses (Bates et al., 2010b) as primary producers, accompanied by a variety of chemotrophic bacteria (da Rocha et al., 2015), archaea (Soule et al., 2009), and fungi (Bates et al., 2010a). Biocrusts are ecologically important biotic components of arid lands (see reviews by Eldridge and Greene, 1994; Belnap et al., 2016), but they can occur in a variety of climatic regions, colonizing places where sunlight reaches the soil surface or even temperate areas subject to disturbance (Gundlapally and Garcia-Pichel, 2006; Elbert et al., 2009; Pointing and Belnap, 2012). Cyanobacteria contribute to important soil functions within biocrusts, providing stability and protection against erosive forces (Belnap, 2003, 2005) and are also responsible for carbon and nitrogen fixation, enriching soils with macronutrients (Johnson et al., 2007) and micronutrients (Beraldi-Campesi et al., 2009). These contributions of cyanobacteria to the ecosystem highlight the importance of understanding their composition and function worldwide.

However, a majority of the studies on cyanobacteria have focused in arid or semiarid areas in North America (Garcia-Pichel et al., 2013; Couradeau et al., 2016; Fernandes et al., 2018), China (Zhang et al., 2016), Australia (Delgado-Baquerizo et al., 2018), Spain (Williams et al., 2016), and the Middle East (Abed et al., 2010; Hagemann et al., 2015; Nejidat et al., 2016). The bundle-forming morphogenus *Microcoleus* seems to be dominant worldwide, even in areas where studies only used microscopy or clone libraries, such as Chile (Baumann et al., 2018) and the hyperarid Atacama Desert (Patzelt et al., 2014). Arid land biocrusts are usually first colonized by *Microcoleus* species, which stabilize the soil and start fixing carbon (Garcia-Pichel and Wojciechowski, 2009), enriching the soil and allowing heterocytous nitrogen-fixing cyanobacteria, usually *Scytonema* sp. and *Tolypothrix* sp. to colonize. Other common cyanobacteria present in arid land crusts include species of *Nostoc*, *Calothrix*, *Chroococidiopsis*, *Leptolyngbya*, *Phormidium* and *Schizothrix*. Whenever compositional studies have been carried out in biocrust from other climates, apparently differentiated communities were encountered (Pushkareva et al., 2015; Muñoz-Martín et al., 2019).

In Brazil, cyanobacterial biocrusts are found in both arid and semiarid areas in the Northeast of the country, but also in the savanna biome called *cerrado*. This biome represents about 2 million km^2 or 23% of the country's land surface, surpassed only by the Amazonian forest (Ratter et al., 1997), but its natural areas (anthropized areas – urban and agricultural – and water bodies excluded) cover approximated 1.24 million km^2 (Sano et al., 2007). Some of *cerrado* phytophysiognomies are more favorable to crust occurrence due to the absence of a developed canopy and these non-forest formations are estimated to cover some 0.8 million km^2, corresponding to 68% of the total *cerrado* area, according to Sano et al. (2007). The *cerrado* climate is classified as semi-humid tropical and is one of the most humid savanna regions in the world but with a severe dry season during April-September. Average annual precipitation varies between 800 and 2,000 mm, and average annual temperatures between 18 and 28°C (Eiten, 1982). Preliminary analyses by microscopy revealed the possibility of finding species not previously reported for biocrusts. Considering the absence of prior biocrust work on the *cerrado*, we undertook a survey of six sites to study their cyanobacterial components. The results of such an approach allowed us to compare the biodiversity and composition of the cyanobacterial assemblages of crusts from different environments (savanna vs. desert). In addition, the influence of selected environmental factors on composition within *cerrado* biocrusts was also evaluated. Our study provides a necessary foundation for more detailed studies and a relevant source of information for management and restoration practices of disturbed areas (Giraldo-Silva et al., 2018), particularly in view of their compositional uniqueness, which makes extrapolation of knowledge obtained from desert biocrust uncertain.

MATERIALS AND METHODS

Sampling

Biocrusts were sampled in six sites distributed in four preserved areas in the SE region of Brazil (**Supplementary Figure S1**): *Furnas do Bom Jesus* São Paulo State Park of (one site), *Vassununga* São Paulo State Park (one site), *Serra do Cipó* National Park (two sites: *Cipó* and *Capão*), and *Serra da Canastra* National Park (two sites: *Canastra* and *Zagaia*) (**Table 1**). Ten sequential equidistant samples were collected throughout a 200 m long transect with two parallel transects set at each site, encompassing a total of 120 samples for the study (10 samples/transect × two transects/site × six sites). Soil crusts were collected with a Petri dish (55 mm × 1 cm high) and transported to the laboratory where they were kept dry at −20°C until processing.

Library Preparation and Illumina Sequencing of the 16S rRNA Gene

Soil grains, rocks, and organic matter (plant roots and leaves) were manually removed, and 1 mg of the sampled biocrust was

TABLE 1 | Samples and sampling localities of biological crusts in *cerrado* savanna.

Sample code	Origin	GPS coordinates
FU1, FU2, FU3, FU4, FU5, FU6	*Furnas do Bom Jesus* State Park	20°14′S, 47°27′W
CI1, CI2, CI3, CI4, CI5, CI6	*Serra do Cipó* National Park – *Cipó*	19°20′S, 43°34′W
CP1, CP4, CP2, CP5, CP3, CP6.	*Serra do Cipó* National Park – *Capão*	19°20′S, 43°34′W
CA1, CA2, CA3, CA4, CA5, CA6	*Serra da Canastra* National Park – *Canastra*	20°21′S, 46°38′W
ZA1, ZA2, ZA3, ZA4, ZA5, ZA6	*Serra da Canastra* National Park – *Zagaia*	20°21′S, 46°38′W
VA1, VA2, VA3, VA4, VA5, VA6	*Vassununga* State Park	21°37′S, 47°37′W

used for DNA extraction with MoBio Powersoil kit (Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The region V3-V4 of 16S rRNA gene (420 bp) was amplified with CYA 359 and 781a/b primers as described in Nübel et al. (1997), with an overhang Illumina adapter included in the primers. The PCR reaction contained 10 ng of eDNA, 0.2 μM of each primer, and 1x KAPA HiFi HotStart Ready Mix (KAPA Biosystems) to 25 μl of final volume. The PCR product from the samples 1–3, 4–6, and 7–10 of each transect were pooled and purified using AMPure XP purification kit (Beckman Coulter Inc., Brea, CA, USA). Afterwards, Illumina sequencing adapters and dual-index barcodes were added to the amplicon target using the Nextera XT Index Kit (Illumina, USA), according to the manufacturer's instructions. The product was purified using AMPure XP purification kit, quantified with Qubit Fluorometric Quantitation (Thermofisher/Life Technology, USA). The samples were normalized and, then, pooled in an equimolar fashion. The preparation of the samples followed the Illumina guidelines for sequencing in a MiSeq platform (Illumina) available at Center of Nuclear Energy and Agriculture (ESALQ/USP) and using MiSeq Reagent kit v3. 2 × 300 cycle.

Data Analysis Pipeline

The 16S rRNA gene forward and reverse sequences were paired using PANDAseq (Masella et al., 2012) and a fastq file was generated. The "QIIME 1.9" [Quantitative Insights into Microbial Ecology (Caporaso et al., 2010)] was used for further analyses. The script "multiple_split_libraries_fasq.py" was run without quality filtering, as quality filtering was done before pairing using Trimmomatic (Bolger et al., 2014). Next, the script "pick_open_reference_otus.py", clustered reads into 97% self-similar operational taxonomic units (OTUs) using SUMAClust (Schloss, 2016) and SortMeRNA (Kopylova et al., 2012) in combination with the Greengenes 13_8 database (DeSantis et al., 2006). A filter was applied to the OTU table and only the OTUs that appeared in at least two samples were considered in the analyses. The OTU table also was used to do a rarefaction curve using "Chao1" and "goods_coverage" methods, through the script "alpha_diversity.py", also in "QIIME 1.9". All sequence datasets are publicly available through NCBI under the project "Diversity and ecology of cyanobacteria of biological soil crusts in Brazilian Savannah" (NCBI identification number: SRP137259).

Operational Taxonomic Unit Taxonomic Assignment

OTUs were identified by comparison with taxonomic information provided by public databanks. OTUs that presented more than 199 reads were first identified using QIIME (based on SortMeRNA and the Green Genes 13_8 database – DeSantis et al., 2006) and then compared one by one with GenBank data (NCBI nr/nt) using the tool "Basic Local Alignment Search Tool" (Baumann et al., 1990) to refine the initial identification. Cyanobacterial OTUs' taxonomic assignment at the genus and species level was further informed through phylogenetic placement in the cyanobacterial reference database, Cydrasil (v. rc1, https://github.com/FGPLab/cydrasil). The Cydrasil rc1 database contains 1,161 curated cyanobacterial 16S rRNA gene sequences that are at least 1,100 bp long and includes a phylogenetic tree generated using RAxML 8 (Berger and Stamatakis, 2011). Query cyanobacterial sequences were aligned to the reference alignment with PaPaRa (Berger and Stamatakis, 2011), placed into the reference tree using the RaxML8 Evolutionary Placement Algorithm (Berger and Stamatakis, 2011) without changing the tree topology, and visualized on the iTOL 3 server (Letunic and Bork, 2016). This procedure allowed to relate an OTU sequence to a well-curated specific phylogenetic group or clade in a database that is enriched in cyanobacterial sequences from biocrusts, and thus confirm (or correct) the initial taxonomic identification. In general, OTUs that were 95% similar to an identified sequence (following Yarza et al., 2014) and placed in a highly supported and well-defined clade composed of coherently identified sequences were considered pertaining to the same genus. OTUs that presented less than 94% of similarity to the closest sequence were not identified. OTUs related to a single genus name but distributed in different clades (as the cases of the polyphyletic genera *Leptolyngbya* and *Microcoleus*) were considered to be effective genus-level taxa and given provisional identifiers (e.g., *Leptolyngbya* - Clade I, *Leptolyngbya* - Clade II…). To facilitate the visualization of the OTUs distribution and the composition of the sites, plots were constructed using the packages "ggplot2," "scales" (Wickham, 2018a,b) and "reshape2" (Wickham, 2017) written in R language (R Core Team, 2018).

Comparison of the Taxonomic Composition Between Regions

A meta-analysis of a set of 15 libraries of bacterial 16S rRNA gene sequences of biocrusts from North American deserts (**Table 2**; Velasco Ayuso et al., 2017: NCBI identification numbers PRJNA343817; Fernandes et al., 2018: NCBI identification number PRJNA394792) was used for comparisons. These libraries had been constructed using primers (515F and 806R primers - Caporaso et al., 2012), whereas those in this work used CYA 359 and 781a/b (Nübel et al., 1997). Because of this, the quality-controlled and paired sequence files were merged into a single FASTA file and imported together into the QIIME 22018.2 for analyses.

TABLE 2 | Samples and sampling localities of biological crusts in United States of America deserts used for comparison.

Sample code	Origin	GPS coordinates
11, 12	Sevilleta Long Term Ecological Research, Chihuahuan desert	34°20′N, 106°41′W
14, 17, 20, 24, 28, 30, 31, 34, 36, 40	Sevilleta Long Term Ecological Research, Chihuahuan desert	34°33′N, 106°72′W
Jornada	Jornada Long Term Ecological Research, Chihuahuan desert	32°54′N, 106°72′W
Fort Bliss	Jornada Long Term Ecological Research, Chihuahuan desert	32°43′N, 105°98′W
Burr	Hill Air Force Base, Great Basin Desert	41°10′N, 113°00′W

Sequences were clustered at 97% similarity using closed reference OTU picking with VSEARCH (Rognes et al., 2016) with the Green Genes 13_8 database (DeSantis et al., 2006) providing the reference sequences. The resulting OTU table was filtered to only include cyanobacterial sequences. OTUs were then aligned using Mafft (Katoh and Standley, 2013) and a phylogenetic tree was generated using FastTree (Price et al., 2010). Community differences were assessed *via* permutational multivariate analysis of variance (PERMANOVA) performed on Bray-Curtis distance matrices of relative abundance derived from sequencing and used 9,999 permutations. PERMANOVAs were performed using the function "adonis2" in the <vegan> package (Dixon, 2003) run in "R" (R Core Team, 2018). The <vegan> function "betadispar" was used to test the variances (PERMDISP). A p of 0.05 was set as the significant threshold for all multivariate statistical analyses. Community composition was visualized with NMDS, using 25 restarts and 9,999 iterations.

Identification of Significant Environmental Parameters

Climatic data were obtained from the public database available at Center for Weather Forecasting and Climate Research (INPE/MCT; http://bancodedados.cptec.inpe.br/). Average annual precipitation (PRE), average annual high temperature (HT), average annual air humidity (AH), and altitude (ALT) were retrieved. Soil temperature (ST) and pH, which were measured in the field, completed the environmental dataset (**Table 1**). The packages "vegan" (Oksanen et al., 2016), "ggplot2" (Wickham, 2018b), and "gridBase" (Murrell, 2014), written in R language (R Core Team, 2016) were used to relate environmental data to the distribution and abundance of OTUs. After normalizing the OTU table, a redundancy analysis (RDA) was run as a constrained ordination method to search for possible spatial patterns in the cyanobacterial database.

RESULTS

Cyanobacterial Diversity From *cerrado* Biocrusts

Altogether, we detected 14,465 cyanobacterial OTUs, of which the 600 most frequently represented comprised 70% of total reads. Rarefaction curves showed that most samples (except CA1, ZA3, and ZB1) reached a plateau, and therefore most of diversity was accessed (**Supplementary Figure S2**). The samples that did not reach the plateau were still included in the subsequent analyses because they were similar in community composition with other samples from the same localities that did.

With our taxonomic identification constrained at the genus level, a large portion of the cyanobacterial diversity remained unassigned (*Canastra* 34.5%, *Capão* 19.8%, *Cipó* 46.5%, *Furnas* 9.4%, *Vassununga* 26.9%, and *Zagaia* 35.9%). But a majority of the unassigned OTUs also failed to align to sequences found within the NCBI database. Even among publicly available environmental sequences, such OTUs did not align with greater than 95% sequence identity, speaking for the presence of a significant level of biodiversity novelty in our biocrusts. At this level of resolution, community composition was relatively homogeneous among samples from the same site (**Figure 1**). OTUs assignable to various clades of *Leptolyngbya* had the most reads. They were dominant in *Furnas*, *Cipó*, *Capão*, and *Canastra*. In some *Capão* samples, sequences allied to *Pycnacronema* were also abundant. The most compositionally divergent communities within our set were those from *Zagaia* and *Vassununga*, where biocrusts were dominated by sequences assignable to *Porphyrosiphon notarisii* Kützing ex Gomont.

On the basis of OTUs (i.e., independently of taxonomic assignments and including unassigned OTUs), communities also differed significantly in composition (**Supplementary Figure S3**) among localities but not within them (PERMANOVA, Pseudo-F = 5.6904, df = 5/30, $p < 0.0001$), each locality being statistically different from each other in pairwise comparisons ($p < 0.05$). Again here, *Zagaia* and *Vassununga* seemed to be the most divergent *cerrado* localities along a compositional continuum. These differences, however, were much less marked than those found between North American desert communities and those from the *cerrado* as a whole (**Figure 2**; PERMANOVA, Pseudo-F = 16.442, df = 1/50, $p < 0.0001$).

Identity of Major Cyanobacterial Members in the *cerrado* Biocrusts

The detailed phylogenetic placement using Cydrasil revealed the occurrence of several important clades of OTUs, which we describe in more detail here. The placement of major *cerrado* biocrusts inhabitants within the cyanobacterial radiation is given in **Figure 3**. Details for the different taxa are in **Supplementary Figures S4–S6**.

Leptolyngbya Clade I fell within the poorly defined, polyphyletic complex of sequences belonging to thin filamentous cyanobacteria that are usually attributed to this morphogenus and was formed by OTUs related to the public sequence of *Leptolyngbya frigida* (Fritsch) Anagnostidis and Komárek. It was clearly polyphyletic to the clade containing the sequence for the generic type species (*L. boryana* Anagnostidis and Komárek), so it likely represents a new generic unit. A second clade in this complex, *Leptolyngbya* clade II, was composed of OTUs similar to the sequence of *Leptolyngbya* sp. 7FUR (MF109116), but they were also, as *Leptolyngbya* Clade I, polyphyletic to true *Leptolyngbya*. Finally, OTUs placed within *Leptolyngbya* Clade III gathered around the sequence of the type species and are likely *Leptolyngbya sensu stricto*. A tree for the "*Leptolyngbya* complex" with assignments can be found in the **Supplementary Figure S4**.

A set of abundant OTUs with high representation in some of our localities was affiliated with *Porphyrosiphon notarisii* as judged by close similarity with several new sequences derived from *bona fide* cultures obtained from *Furnas* biocrusts (now permanently added to the Cydrasil database). This clade of sequences is well separated phylogenetically from sequences of other large-celled members of the Phormidiaceae, indicating that they do indeed represent a differentiated generic entity, as the traditional taxonomy would predict (**Supplementary Figure S5**).

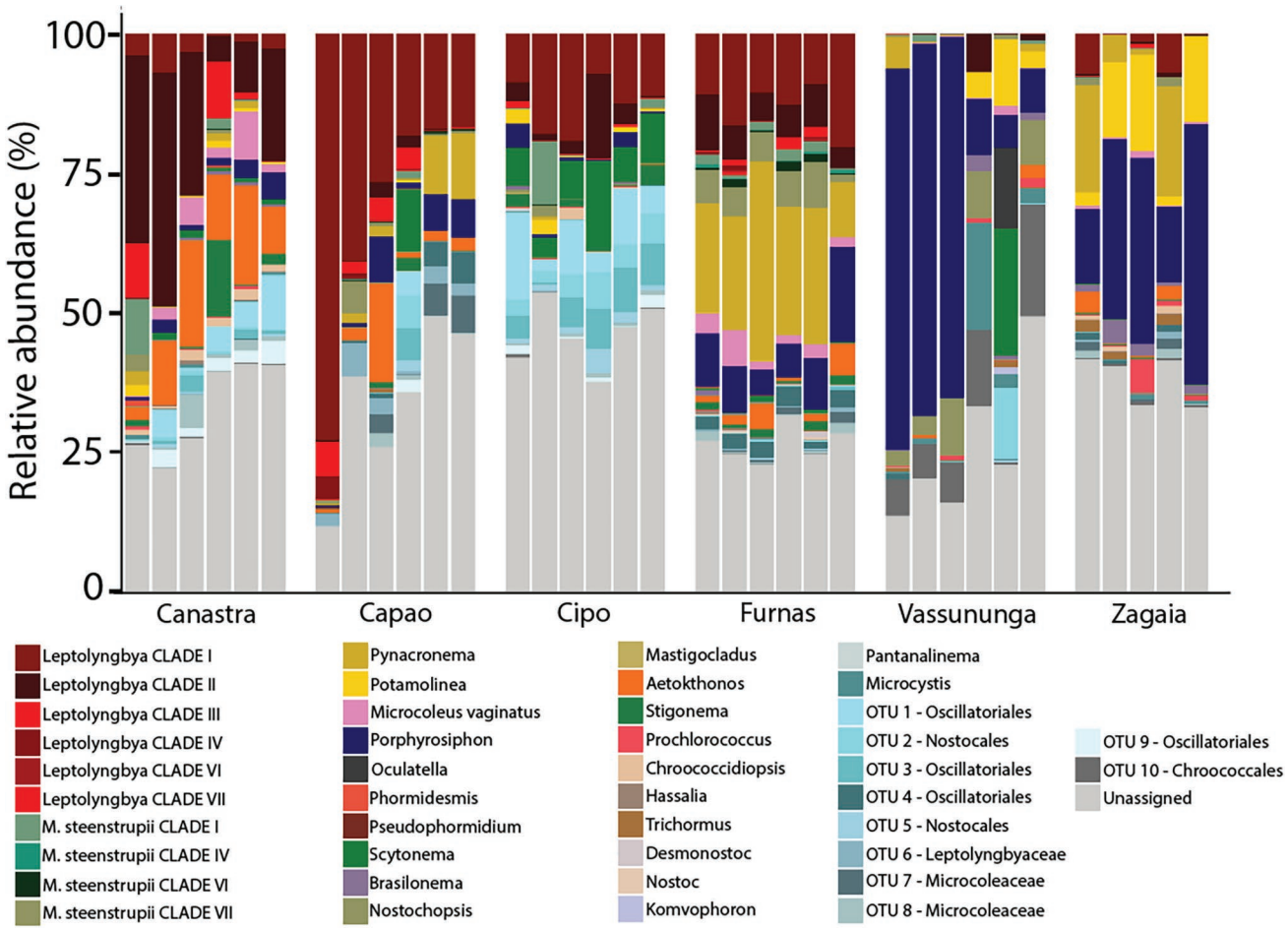

FIGURE 1 | Relative abundance by proportion of 16S rRNA gene sequence reads showing assignable and unassignable to the genus-equivalent level in *cerrado* biocrusts, grouped by sampling locality.

A second complex of biocrust cyanobacteria corresponds to the epithet "*Microcoleus steenstrupii*" which has been recognized as a supra-generic entity in need of re-evaluation, not only because its members are not related to *M. vaginatus* (Vaucher) Gomont ex Gomont (the type species for the genus *Microcoleus*, which falls within the Complex Filamentous clade of **Figure 3**), but also because it encompasses a variety of separated clades with apparently diverging ecological traits (Fernandes et al., 2018). *Sensu lato*, the complex also includes sequences aligned with cultured strains assigned to *M. paludosus* Gomont, and the recently described *Pycnacronema savannensis* Martins, Machado-de-Lima, and Branco isolated and described from Brazilian savanna biocrusts (Martins et al., 2019). All these cyanobacteria are morphologically indistinguishable from *Phormidium*, except for the fact that they often form trichome bundles. Many OTUs in our crusts clustered within this complex, and given the difficulty in systematics, we have maintained the epithet "*Microcoleus steenstrupii* complex" to refer to them, except for OTUs clearly affiliated with *P. savannensis*, which conformed a major component in some of our crusts (**Supplementary Figure S5**). Several new full sequences of *P. savannensis* from *bona fide* cultures were included in the database before analyses to ensure that this choice was correct. OTUs in this complex not affiliated with *Pycnacronema* were not very numerous in terms of total reads.

OTUs most similar to the sequences of *Microcoleus vaginatus*, the type species for the genus *Microcoleus* in the Oscillatoriaceae, and likely the most common terrestrial cyanobacterium globally (Garcia-Pichel, 2009), while not very common here, constituted another clear assignment.

Within the heterocytous cyanobacteria, many OTUs were phylogenetically cognate with members of *Brasilonema* sp. (**Supplementary Figure S6**), previously unreported from soil crusts. Among the three major heterocytous types found in North American arid land crusts (Yeager et al., 2007), only *Scytonema* found significant representation in the *cerrado* crusts.

Relationship Between Operational Taxonomic Units and Environmental Variables

Our RDA analyses (**Figure 4**) showed that the combined effect of all environmental variables considered (**Supplementary Table S1**)

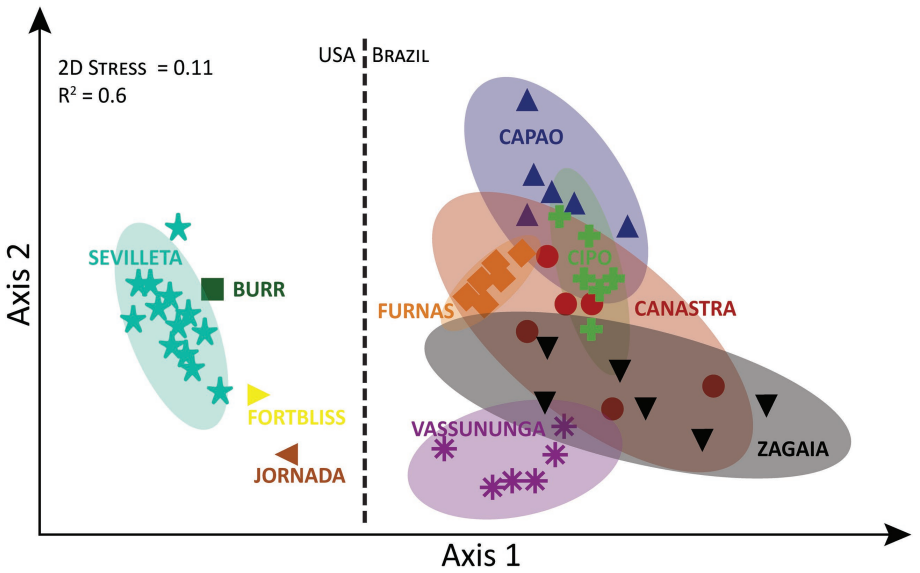

FIGURE 2 | Non-metric Multi-Dimensional Scaling of biocrust cyanobacterial community composition at the OTU level, based on Bray-Curtis similarity including *cerrado* samples and literature-based data for North American biocrusts, for comparison. Ellipses correspond to 95% CI spaces. Data points and ellipses are color coded by locality.

could explained 58% of the total variability in cyanobacterial community composition among Brazilian biocrusts. The two statistical axes represented in **Figure 4** explained 40% of this variability. The opposing vectors of high annual temperature (HT) and precipitation (PRE)/air humidity (AH) explained the majority of the variation in community composition among locations, particularly separating *Zagaia* and *Vassununga* (related to hotter, drier climate) from the rest. The arid end of this *continuum* was determined significantly by the strong contributions of *Porphyrosiphon*, the opposite end by those of *Leptolyngbya* Clade I. Variability among *Canastra*, *Cipó*, *Capão*, and *Furnas* communities seemed to be driven by soil pH (**Figure 4**).

DISCUSSION

Microcoleus vaginatus, *M. steenstrupii* Petersen, *M. sociatus* West and West, *Nostoc commune* Vaucher ex Bornet and Flahault, *Calothrix*, *Lyngbya*, *Oscillatoria*, *Phormidium*, *Scytonema*, and *Tolypothrix* are considered the most representative and abundant taxa of cyanobacteria in biological soil crusts throughout the world (Büdel, 2003). *Microcoleus vaginatus* and the "*Microcoleus steenstrupii* complex" are dominant in biocrust from North American and other arid lands (Garcia-Pichel et al., 2001; Gundlapally and Garcia-Pichel, 2006; Büdel et al., 2009; Hagemann et al., 2015; Schulz et al., 2015; Dulić et al., 2017). Interestingly, many of those taxa were absent (or present only in low abundance) in the *cerrado* crust. Instead, *Leptolyngbya* and *Porphyrosiphon* were well represented and often dominant in these subtropical biocrusts. Among the heterocytous cyanobacteria, *Nostoc* sp. and *Tolypothrix* sp., typical of the colder climates in arid lands (Yeager et al., 2012; Zhou et al., 2016; Giraldo-Silva et al., 2019, submitted) had no relevance in the *cerrado* crusts, yielding to *Scytonema* and *Brasilonema*. Additionally, many of the cyanobacterial OTUs from this study did not have phylogenetically close representatives within common databases, resulting in a high proportion of unassigned diversity. This indicates that a significant unique component of biodiversity in the *cerrado* remains to be characterized. The notion of compositional idiosyncrasy of the *cerrado* biocrusts is supported strongly by their clear differentiation from arid land biocrusts in nMDS analyses. One cannot principally distinguish from these data if barriers to dispersal or differential selection by environmental conditions are responsible for the pattern. However, the fact that at least some taxa typical of arid land crusts are found at low population density in the *cerrado* would rather support the latter view.

Leptolyngbya has been commonly reported in BSCs but rarely appearing as the dominant genus in biodiversity studies (Kaštovská et al., 2005; Alwathnani and Johansen, 2011). It is distributed worldwide (Büdel et al., 2009; Hagemann et al., 2015; Schulz et al., 2015), from glacial areas in Svalbard (Norwegian archipelago – Kaštovská et al., 2005) to desert areas at mid-latitudes (Mojave Desert - Alwathnani and Johansen, 2011). However, it is unclear to which of its many polyphyletic clades each reported occurrence belongs. Likely, the "lumping" morphological approach to this genus conceals significant biological and phylogenetic diversity rather than support the notion that *Leptolyngbya* is a true generalist organism. Based on the results and analyses presented here, separating *Leptolyngbya* sequences into distinct phylogenetic clades may help solve this issue and allow the establishment of an ecologically meaningful systematic treatment.

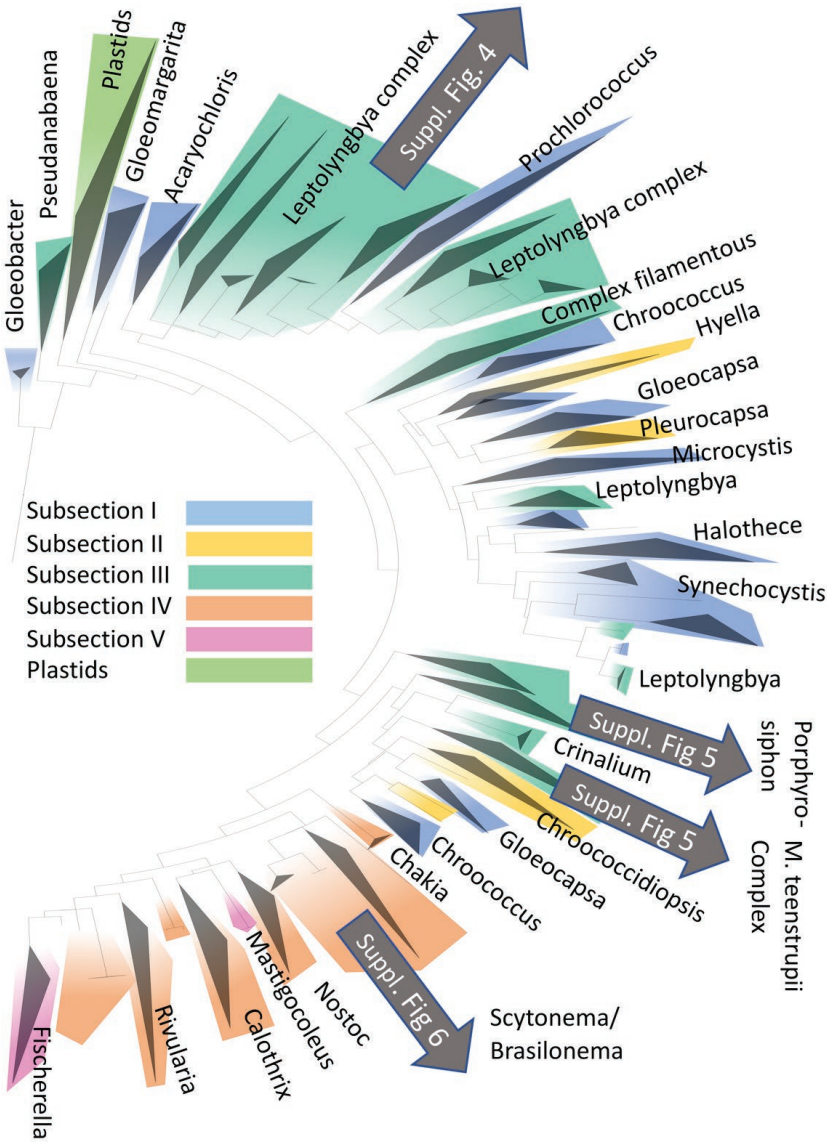

FIGURE 3 | Placement of the more abundant cyanobacteria found in the present study (with greater number of 16S rRNA gene sequence reads; gray arrows) in the phylogenetic radiation of cyanobacteria based on the Cydrasil database. Clades have been colored according to Bergey's Manual of Determinative Bacteriology's major morphotypic subsections, and exemplary genera contained within selected clades have been used to label them. Detailed phylogenies of biocrusts OTUs can be found in **Supplementary Figures S4–S6**, as indicated.

The recognition of this distinct composition and the high frequency of *Porphyrosiphon* and *Leptolyngbya* clearly speak for the need of investigating these players at the physiological and ecological levels since they will be paramount in establishing a baseline for future understanding of the dynamics of the *cerrado* environment. On the assumption that emergent ecological properties of biocrusts depend on their species composition, it will be necessary to revise locally the paradigms of biocrust function and ecosystem services that have largely been derived from arid land biocrusts. *Porphyrosiphon*, an easily recognizable morphotype because of its bright red sheaths, e.g., has also been reported from biocrusts of the savanna ecosystems of Australia (Williams and Büdel, 2012) and Africa (Ullmann and Büdel, 2003), where it is considered widely dominant over other cyanobacteria. We could relate the presence of this organism in the field with sequences of cultivated isolates, which should enable an easier identification of its global biogeography in the future. *Porphyrosiphon* produces gelatinous sheaths that could bind soil particles playing a similar role to that of bundle-forming *Microcoleus vaginatus* and *M. steenstrupii* in arid land biocrusts (Péli et al., 2011).

OTUs that could not be assigned to genus level but with high number of reads, and for which a sufficiently similar

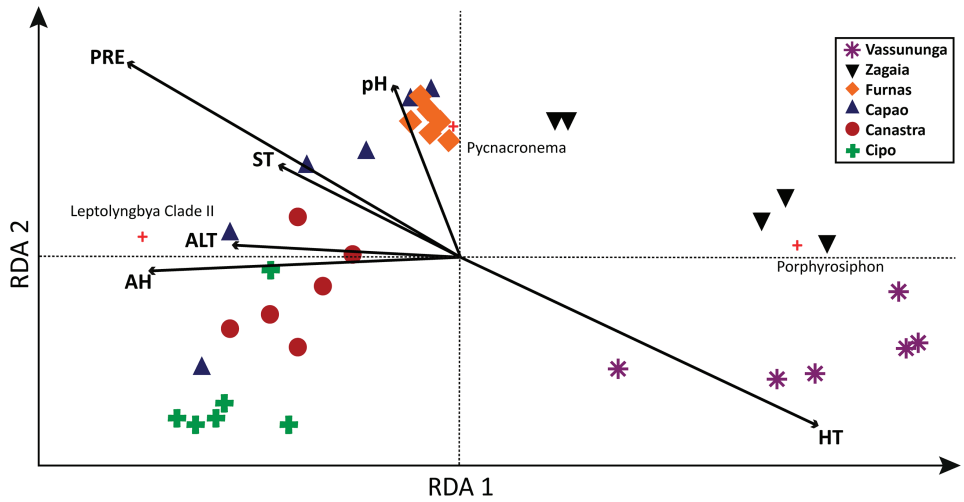

FIGURE 4 | Redundancy analysis (RDA) relating climatic variables with cyanobacterial diversity in 36 sites from six localities in the Brazilian *cerrado*. ALT, altitude; AH, air humidity; PRE, average annual precipitation; ST, pH, soil temperature; HT, average annual maximum temperature.

sequence was found to public database entries, were classified at order rank (OTU1 to OTU10). This was preferred (instead of family rank) because familial divisions in *Cyanobacteria* are phylogenetically ill-defined or polyphyletic, and taxonomically unresolved. Most unassigned OTUs, however, represent sequences with low number of reads.

The importance of aridity (including temperature and rainfall) as determinant of variations in species composition within the *cerrado*; however, parallels what has been found in arid land and Mediterranean biocrusts (Garcia-Pichel et al., 2013; Muñoz-Martín et al., 2019). Although it is necessary to consider that the set of variables used in this work was rather restricted, in the present case, *Leptolyngbya Clade*-I abundance was positively correlated with the wetter, least hot sites, while *Porphyrosiphon* showed an opposite trend, explaining their apparent mutual exclusion as dominant forms. *Porphyrosiphon notarisii* was found to be also a dominant species in Australian Mulga Lands (Williams and Büdel, 2012), where it reportedly tolerated and recovered from drought exceptionally well. This success could be, at least in part, related to their copious sheath investments, which are thick and deeply colored with a red extracellular pigment known as gloeocapsin, likely serving a sunscreen role, and which probably decreases the albedo of the soil, increasing its temperature even further (Couradeau et al., 2016).

The biodiversity of cyanobacteria that exists in biocrusts from *cerrado* seems to be distinct from that of other well-known assemblages, such as those from deserts, and in many aspects quite unique. Dominant species in *cerrado* crusts are marginally present, or even absent, in arid lands evidencing the forcing by large-scale climatic patterns. At the same time, biocrusts from relatively close areas within the *cerrado* domain showed variations in composition that are attributed to local, geographically more restricted conditions. The interaction of the conditions on both scales determines the specific characteristics of the communities and may have implications for the ecological services that biocrust may be able to provide to the ecosystem in each locale.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the NCBI Bioproject database under the accession number PRJNA381019 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA381019).

AUTHOR CONTRIBUTIONS

NM-L and VF contributed equally to this manuscript. NM-L, JR, and LB designed experiments and sampled the biocrusts. NM-L, JR and VF performed the laboratory research. NM-L, VF, FG-P and LB processed and analyzed the data. DR wrote and executed the codes for bioinformatics analysis. SV wrote the code for the environmental data analysis and executed it. NM-L, VF, FG-P and VF wrote the manuscript. All authors contributed to the discussion of data and finalization of the manuscript. All authors read and approved the final 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.2019.02798/full#supplementary-material

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Components and Predictors of Biological Soil Crusts Vary at the Regional vs. Plant Community Scales

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Although biological soil crusts (biocrusts) occur globally in arid and semi-arid environments, most of our knowledge of biocrust cover and ecology is from a relatively small number of locations worldwide. Some plant communities are known to have high cover of biocrusts, but the abundance of biocrusts is largely undocumented in most plant communities. Using a data driven approach, we identified 16 plant communities based on plant cover from the Assessment, Inventory, and Monitoring Strategy data from the Bureau of Land Management (AIM, 5,200 plots). We found that abundance of lichens and mosses varies among communities, but that both components of biocrusts are present in all plant communities. Biocrusts are indicators of two of these communities: one that is defined by high cover of mosses and basin big sagebrush and one that is defined by high cover of lichens and shadscale saltbush. Using non-parametric multiplicative regression, we evaluated a suite of abiotic and disturbance variables to assess the degree to which climate and soils are associated with the abundance of lichens and mosses at the regional scale. At the regional scale, soil depth and maximum vapor pressure deficit were found to be strongly associated with the abundance of lichens and January minimum temperature dictated the abundance of mosses. At the scale of plant communities, community specific metrics of soils and climate were better able to explain the abundance of biocrusts. Our demonstration of the presence of biocrusts across the western US suggests that studies on ecosystem function could include these organisms because they are present in all plant communities, maintain arguably stronger associations with climatic variation, are directly associated with soils, and contribute to ecosystem functions that are not solely maintained by vascular plants.

Keywords: AIM data, biocrusts, climate, disturbance, lichen, moss, non-parametric multiplicative regression, soils

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SIGNIFICANCE

Biological soil crusts (biocrusts) contribute to ecosystem functions such as carbon and nitrogen cycling, hydrologic cycling, soil stabilization, and maintenance of albedo in arid and semi-arid environments worldwide. Most studies on biocrusts are from single plant communities in a few locations. The occurrence of biocrusts has not previously been related to plant communities at the regional scale of the western United States (US). We demonstrate that biocrusts occur in all plant communities but with varying abundance across the semi-arid and arid western US. Regionally, moss abundance peaks on lands with January minimum temperatures between 5 and 0°C. Lichen abundance peaks where soil depth is between 0 and 50.8 cm and when annual maximum vapor

pressure deficit falls between 15 and 40 hPa. At the scale of plant communities, site-specific factors are generally stronger predictors of abundance of mosses and lichens compared to the regional drivers. These results highlight that biocrusts occur in all plant communities across the semiarid and arid western US and suggest that if management goals include ecosystem functions to which biocrusts contribute, biocrusts could be included.

INTRODUCTION

Globally, interspaces among vascular plants in undisturbed semi-arid ecosystems are occupied by biological soil crusts (biocrusts), referring to lichens, mosses, and cyanobacteria that grow on the soil surface (Belnap and Lange, 2001). Many of these organisms contribute to nutrient and hydrologic cycling, dust capture, and all of them contribute to the prevention of soil erosion (Evans and Ehleringer, 1993; Belnap and Gillette, 1998; Reynolds et al., 2001; Barger et al., 2006; Bowker et al., 2011). The occurrence of biocrusts is included in the larger description of biocrusts of North America by Rosentreter and Belnap (2001), highlighting that biocrusts occur in the hot deserts, cold deserts, and chaparral. In the absence of disturbance, where space between plants is large, the abundance of biocrusts increases with increasing moisture availability (Bowker et al., 2006; Büdel et al., 2009; Kidron et al., 2010). However, drivers behind the occurrence and abundance of biocrusts at the biogeographic scale are largely unknown (Steven et al., 2013). This is likely because biocrusts have not been studied in the same way that plant communities have been identified, with studies that span regions but identify areas that are homogenous regarding driving factors of soils and climate. Studies that intersect the occurrence of both biocrusts and plant communities are rare and generally within a single plant community (Hilty et al., 2004; Reisner et al., 2013; Warren et al., 2015; Condon and Pyke, 2018a,b), making generalizations difficult regarding drivers of biocrust abundance. To date, we do not know of any studies on occurrence and abundance of biocrusts that span plant communities at the regional scale of the western US that would allow for the identification of drivers of biocrust occurrence and abundance. Working across scales would allow for a demonstration of the degree to which biocrusts are specific to a particular plant community vs. the degree to which they may be ubiquitous across communities of the western US.

Soils and climate allow plant species to grow in a given location but interactions amongst species, and interactions between species and disturbances, can filter or promote occurrence (Gunderson, 2000). Soils and long-term averages in precipitation are important for determining plant species occurrence and plant communities (Barbour and Billings, 2000). However, it is likely that climatic and edaphic factors that dictate extreme conditions are favoring biocrusts, particularly the lichen component (Hale, 1967). Lichens and most mosses lack protective cuticles, vascular structure and water-transporting roots, leaving them dependent on their surrounding environment for resources (Proctor and Tuba, 2002; McCune and Geiser, 2009). However, they are also poikilohydric, using water as it is available, and with the ability to survive in a desiccated state for years (Breuil Sée, 1993; Proctor and Tuba, 2002; Wasley et al., 2006). They are tolerant of freezing conditions (Lenné et al., 2010) as well as temperatures that are too high for many vascular plants (Hale, 1967). Given these dichotomies, we expect that the extreme ends of the vapor pressure deficit spectrum will favor biocrusts. For example, if there is a high likelihood of dew in the winter but conditions are dry enough to be stressful for vascular plants, cover of biocrusts is expected to be high. Similar relationships are expected with edaphic factors. Sites that are stressful for vascular plants regarding chemistry, water availability, or radiation are expected to favor biocrusts (Garcia-Pichel and Castenholz, 1991; Bowker and Belnap, 2008; Zhao et al., 2010; Rajeev et al., 2013).

The current study aims to demonstrate the occurrence and abundance of biocrusts by plant community across arid and semi-arid, non-forested lands. Although we expect that biocrusts have an affinity for temperature extremes and arid areas, we expect drivers of biocrust abundance to differ at the scale of plant communities, since specific ranges of climatic and edaphic conditions are considered in delineating plant communities. We also expect that land uses and soil characteristics at the scale of a plant community will be more similar and therefore are likely to have a greater influence at this scale as opposed to the regional scale. Although biocrusts are tolerant of climatic and edaphic extremes, we expect that they are less tolerant to disturbances of the soil surface (Condon and Pyke, 2018a,b; Duniway et al., 2018). Under this scenario, soils that are armored with gravel and rocks will be protected from disturbances that perturb the soil surface and are therefore likely to have higher cover of biocrusts. Other driving factors that might influence the occurrence of biocrusts include the affinity of some terricolous lichen species for calcareous soils (McCune and Rosentreter, 2007). We address the following objectives for both the moss and lichen components of biocrusts:

(1) document the occurrence and abundance by plant community across the arid and semi-arid western US, (2) identify drivers of abundance at the regional scale, and (3) compare regional drivers with drivers within each plant community.

METHODS

Identification of Plant Communities

Beginning in 2011, BLM began using a standardized monitoring framework to collect quantitative data that can be used to make management decisions and to meet management objectives of renewable resources such as vegetation, soils, water, fish and wildlife habitat, the Assessment, Inventory and Monitoring (AIM) program (Toevs et al., 2011). These lands are managed for multiple uses including grazing by livestock, energy development, and recreation. A subset of these data, referred to as the Landscape Monitoring Framework (LMF) includes data on plant cover, characterization of soils and disturbance across the western US excluding Alaska. This massive dataset (5,200 locations as of 2015) gives us the ability to assess the occurrence and abundance of biocrusts, specifically mosses, and lichens in multiple biomes across much of the continent.

In the LMF of the AIM program, trained observers visit a randomly assigned location, given specified rejection criteria (see http://aim.landscapetoolbox.org/ accessed 12 May 2017). Rejection criteria for the LMF includes tree cover being >25%, the plot must be <3 miles from the nearest point accessible by vehicle and the land ownership is BLM. If the site is found to be acceptable, observers center two perpendicular 150-foot (45.7 m) transects on the predetermined point. Point-intercept data of plant species and ground cover contacts are recorded for a total of 102 points per plot (Herrick et al., 2005). Point-intercepts from the top of the plant canopy and down to basal intercept can include 5 additional unique intercepts on the way down (for a total of 6 plant intercepts and one ground cover intercept). These possible 7 intercepts are included in our analysis to distinguish communities based on plant composition. For example, Wyoming big sagebrush may be the initial overstory plant intercept species but could have a native or introduced understory. Species taxonomy follows the USDA PLANTS database [United States Department of Agriculture, Natural Resources Conservation Service (USDA, NRCS), 2017] and includes the following non-vascular plant and non-plant categories: none, herbaceous litter, woody litter, artificial litter, organic matter, deposited soil, lichen, moss, rock fragments, and unknown plants. We use all existing data through 2015, resulting in 5200 locations (plots) across 11 states in the western US and summarize data to the plot level. Data are not collected on the occurrence of cyanobacteria and so we do not address the occurrence of cyanobacteria in this analysis.

We use vascular plant species cover data as well as lichen and moss cover to derive plant communities for the region. Plant communities (groups) were formed by hierarchical cluster analysis with indicator species analysis as an objective criterion to select the number of ecologically meaningful groups (Dufrêne and Legendre, 1997). We did not transform cover because we expected that extremes in cover values may strongly favor or discourage lichens and mosses. The most meaningful number of groups demonstrated the highest number of significant indicators (species with $p < 0.05$) and the lowest average p-value across all species (McCune and Grace, 2002). Indicator species analysis considers both the relative abundance of species and the frequency of species within groups. A species cannot receive a high indicator value for a group unless it is both abundant and faithful to that group. Analyses were performed in PC-ORD version 7.

Identifying the Drivers of Moss and Lichen Abundance

We use non-parametric multiplicative regression (NPMR) to select possible drivers of moss and lichen abundances because NPMR naturally handles interactions amongst predictor variables by testing various ranges of predictor variables on ranges of response variables and employing a multiplicative kernel smoother. We use local mean models with a uniform (rectangular) kernel as we expected precipitous changes in cover, moving from areas with high cover to no detected cover. A consequence of the uniform kernel is that response surfaces tend to be relatively rough, as compared to smoother surfaces produced by the more commonly used Gaussian kernel. NPMR allows for the visualization of relationships that might change over a gradient. For example, perhaps moss cover increases with shrub cover to a certain threshold, at which point moss cover starts to decline or plateau at a site. NPMR naturally identifies these values and relationships and can be more accurate compared to linear models when non-linear relationships exist. Models are assessed with a cross-validated R^2 (1- RSS/TSS = xR^2) and overfitting is avoided as response surfaces are estimated for each data point, in the absence of that data point. Overfitting controls were used at the medium level, specifically requiring that a minimum of 10 data points be used in prediction, predictor variables were required to improve xR^2 by 5%, and the minimum average neighborhood size was 0.05 times the number of plots. Sensitivity analysis was performed on each predictor to evaluate its importance relative to other predictors. Conceptually, sensitivity is the mean difference in the response/range in response divided by the difference in predictor/range in predictor in the equation (McCune, 2009):

$$\frac{\left(\sum_{i=1}^{n}\left|\hat{y}_{i+}-\hat{y}_{i}\right|+\sum_{i=1}^{n}\left|\hat{y}_{i-}-\hat{y}\right|\right)}{2n\left|y_{\max}-y_{\min}\right|\Delta}$$

In the above equation, $\hat{y}_{i+}$ is the estimate of the response variable in the case $_i$ having increased the predictor by a small value of Δ and $\hat{y}_i$ is the estimate of the response variable in the case $_i$ having decreased the predictor by a small value of Δ. This calculation for sensitivity gives less weight to large differences and the interpretability is straightforward as a sensitivity = 1 means that a change in the predictor results in a change in the response of equal magnitude (McCune, 2009). We also report the tolerance of each predictor, the half-width of the rectangular. Models with low xR^2 are expected to have low sensitivities and high tolerances for the predictors. It is also possible for a model to have a negative xR^2 if cross validation results in the residual sum of squares exceeds the total sum of squares. Models were run for cover of mosses and lichens across the region as well as within each plant community type.

Soil and Climate Data

NPMR models were run on a subset of data used to define plant communities because soils data were not collected in 2011, resulting in 3,209 plots with soils data. We expected that both lichens and mosses would be highly sensitive to soil characteristics and so we used measured site-specific data as opposed to pre-existing soil maps, which vary in resolution accuracy. Site-specific soils data includes the depth of the surface horizon, the texture of that horizon, the presence of any texture modifier, and effervescence classes which indicate the amount of calcium carbonate in soil, ranging from 1 for not effervescent to 5 for violently effervescent. Soils texture data were made numeric by using the median values of clay, silt, and sand in each texture class. Texture modifiers were ranked by size class as of the modifier and amount. We used PRISM climate data at the 800 m grid scale (http://prism.oregonstate.edu/ accessed 1 May 2018) including 30 year average annual precipitation,

annual maximum vapor pressure deficit, annual minimum vapor pressure deficit, mean dew point temperature in January, average minimum January temperature, average maximum July temperature and elevation. Vapor pressure deficit refers to the difference in the amount of moisture in the air compared with compared with how much moisture the air can hold when saturated in hPa. Dew point is the temperature at which water droplets condense, which varies with barometric pressure and humidity. We anticipated that January temperatures and dew points would be important given the fact that mosses in the semi-arid region of the Intermountain West grow during the winter (Condon and Pyke, 2016) and that mosses often desiccate before freezing (Malek and Bewley, 1978). July is often the hottest month of the year, making July maximum temperature a value that characterizes current temperature extremes (https://www.ncdc.noaa.gov/sotc/national/201707 accessed 13 July 2018). Elevation was also included because in addition to 30 year average annual precipitation, these factors are commonly used to explain occurrence of plant communities (Barbour and Billings, 2000).

Disturbances

AIM data include the occurrence of disturbances at two temporal scales (present and past) as well as two spatial scales (on the plot and the larger area that plot resides in, delineated by fence lines, if present, or 1,000 ft from the plot, United States Department of Agriculture, Natural Resources Conservation Service (USDA, NRCS), 2015). Analyzed disturbances were observed within the management unit (larger area). We coded these as ordinal variables where the disturbance was noted as a "1" if it only occurred in either the past or at present but coded as a "2" if it occurred both in the past and in the present. Disturbances fall under the general categories of land uses, land treatments, and natural features. Disturbance types are described in **Supplementary Table 1**.

RESULTS

A combination of hierarchical cluster analysis and indicator species analysis resulted in 16 plant communities across BLM lands in the western US. This includes one additional group of sites with low cover and as such they did not naturally fit into the other communities. Species that characterized each plant community based on indicator species analyses were used to name the community (**Figure 1**, **Supplementary Table 2**). All plant communities had plots that contained lichens and mosses but with varying abundances of these organisms (**Figure 1**). Two communities are in part defined by cover of biocrusts: big sagebrush-moss and shadscale-lichen because they had high cover of mosses and lichens, respectively.

Most plant communities fit with those described for the Intermountain West by West and Young (2000) with a few exceptions. Field brome—Little Bluestem Grassland and Needle and Thread—Threadleaf Sedge Grassland are tallgrass and mixed grass prairie, respectively (Sims and Risser, 2000). Lesser spikemoss and associates are common of alpine and meadow communities (Billings, 2000) and these species are also common in the grasslands of the northwestern Great Plains of Montana, North Dakota, and South Dakota (Crane, 1990).

At the regional scale of non-forested drylands in the western US, the best predictors of lichen cover were soil depth, maximum vapor pressure deficit and elevation. Areas with generally shallow soils that are drier and sometimes at elevation are more favorable to the lichen component of biocrusts ($xR^2 = 0.215$, **Table 1**, **Supplementary Figure 1**). However, based on xR^2, models that were created for each plant community were generally better predictors of lichen abundance, except for the following plant communities: juniper and the cheatgrass- weedy forb community (**Table 1**). This suggest that factors other than climate and soils, such as past land use, may control the occurrence of biocrusts in these vegetation types, particularly the cheatgrass-weedy forb community which did not result in a useful model. Relationships between soil depth, maximum vapor pressure deficit, elevation and lichen cover are present in many but not all plant communities. To demonstrate the coverage of plots that were classified in each community we show how many plots fell into each plant community, how many were recorded as having mosses and lichens and where each plot was located (**Supplementary Figure 1**).

The best predictors of moss cover at the regional scale were January minimum temperature and presence of insects (**Table 2**). Similar to models predicting lichen cover, models predicting moss cover were generally stronger based on xR^2 when created at the scale of the individual plant community. Exceptions to this include models for the field brome-little bluestem community and the western wheatgrass community (**Table 2**). Relationships between January minimum temperature and moss cover were present in four of the 16 identified plant communities. Additionally, the community with the highest proportion of plots with moss, the big sagebrush—moss community was the only community to have the presence of insects included in its associated model (**Table 2**).

At the scale of plant communities, predictor variables associated with location were common: county, state, latitude and longitude. These factors were significant in nine of the 16 lichens models and 12 of the 16 moss models (**Tables 1**, **2**).

DISCUSSION

We demonstrate that the lichen and moss components of biocrusts occur throughout BLM lands, in all identified plant communities, across the arid and semi-arid western US. The predictors that are commonly used to explain the occurrence of biocrusts work well for the lichen component: drier sites (increased maximum vapor pressure deficit) with shallow soils (soil depth, Rosentreter and Belnap, 2001). However, winter conditions were the best predictors of the abundance of mosses regionally, specifically January minimum temperature. The cover of these components of biocrusts vary by plant community, and the drivers behind the occurrence of both mosses and lichens were generally a combination of soils and climatic factors.

Two plant communities were defined by the abundance of examined biocrust components: big sagebrush—moss

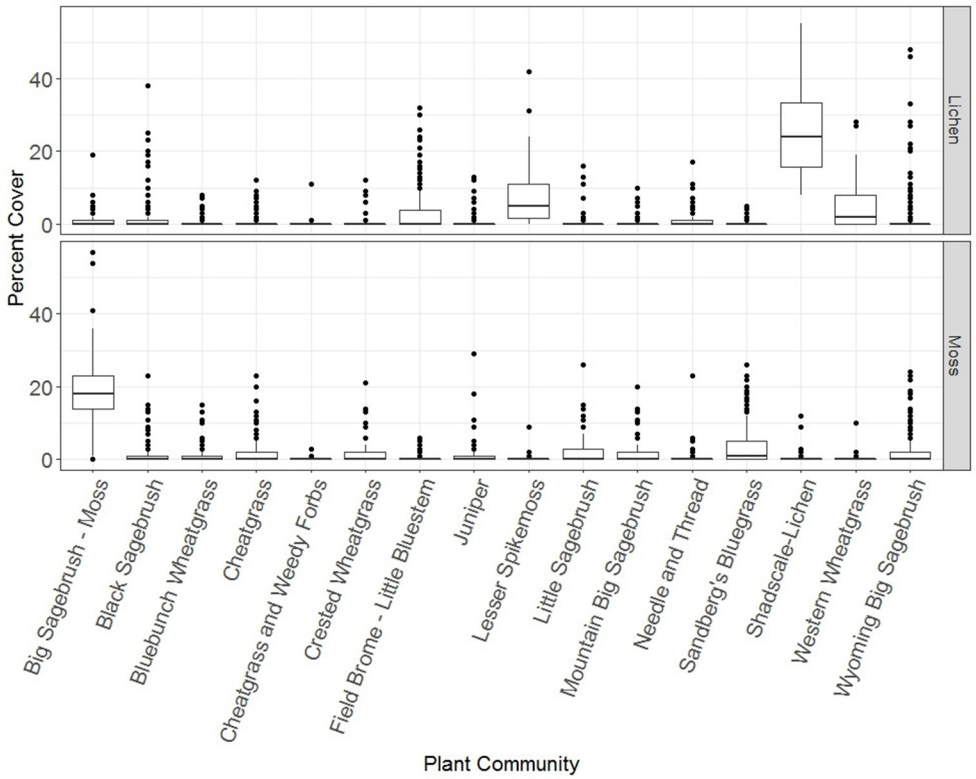

FIGURE 1 | Cover of lichens and mosses within each plant community across the western US summarized as boxplots. Boxplots show the inter quartile range. Whiskers show values within 1.5 the inter-quartile range, the distance between the first and third quantiles. Data beyond this range are plotted as individual points.

TABLE 1 | Best NPMR models for lichen cover by plant community.

Plant community	xR^2	V	N	Tolerances and sensitivities (in that order)
Regional	0.215	14	96.4	**Soil Depth** (3.95, 0.21) **MaxVPD** (1.69, 0.31) Elevation (313.30, 0.11)
Big Sage Moss	0.572	3	10.4	Sand (9.25, 0.06) **County** (16.00, 0.08) **JulyMaxT** (2.74, 0.18)
Black Sage	0.497	1	55.6	**Soil Depth** (2.95, 1.01)
Bluebunch Wheatgrass	0.567	2	8.8	**Latitude** (0.95, 0.52) **Longitude** (0.76, 0.87)
Cheatgrass	0.262	4	19.0	MinVPD (0.49, 0.22) **JulyMaxT** (1.06, 0.25) Vehicle (0.10, 0.00) **Moss** (1.15, 0.63)
Cheatgrass Weedy Forbs*	−0.037	1	16.1	**Soil Depth** (1.70, 0.40)
Crested Wheatgrass	0.906	2	5.4	**Latitude** (0.54, 0.81) **JanDewP** (0.87, 0.15)
Field Brome—Little Bluestem	0.231	2	22.4	**Latitude** (0.83, 0.89) **JanDewP** (0.95,0.27)
Juniper	0.205	2	13.0	**Armor** (0.5, 2.13) **Elevation** (304.75, 0.19)
Lesser Spikemoss	0.383	2	5.9	**JanDewP** (0.19, 1.83) Overhead Lines (0.10, NaN)
Little Sage	0.962	3	5.6	**Longitude** (0.67, 0.48) **JulyMaxT** (1.62, 0.09) Moss (3.90, 0.0001)
Mountain Big Sage	0.554	5	5.2	County (70.20, 0.04) **Latitude** (0.88, 0.33) **Soil Depth** (1.90, 0.32) JanMinT (5.75, 0.08) Prescribed Fire (0.05, NaN)
Needle and Thread—Threadleaf Sedge	0.420	5	8.6	EC (0.20, 0.00) **County** (31.20, 0.24) **Elevation** (226.80, 0.18) Non-rodent (0.10, 0.00) Foot Traffic (1.10, 0.02)
Sandberg's Bluegrass	0.307	4	10.6	**JanMinT** (0.78, 1.17) Roads-dirt (0.10, 0.00) Fire Fighting (1.10, 0.01) **Moss** (10.40, 0.14)
Shadscale Lichen	0.564	3	4.2	Armor (4.40, 0.01) **Latitude** (0.76, 0.81) Livestock (1.10, NaN)
Western Wheatgrass	0.382	3	4.0	**EC** (1.05, 0.26) **MaxVPD** (0.21, 2.15) MinVPD (0.58, 0.11)
Wyoming Big Sagebrush	0.447	3	25.8	**Armor** (2.50, 0.05) **Longitude** (0.82, 0.29) Soil Depth (4.60, 0.04)

*The best two variables in explaining cover as judged by sensitivity are in bold. *No significant model. xR^2, cross validated R^2; V, number of predictors in the model; N, Average neighborhood size. Directions of relationships at the regional scale can be visualized in **Figure 2**. Plot locations for each plant community are shown in **Supplementary Figure 1**. Predictors related to the abiotic environment and disturbance are described in **Supplementary Table 1**. Moss refers to moss cover.*

TABLE 2 | Best NPMR models for moss cover by plant community.

Plant community	xR^2	V	N*	Tolerances and sensitivities (in that order)
Regional	0.148	2	404.2	**JanMinT** (1.39, 0.21) Insect (0.1, 0.00)
Big Sage Moss	0.611	6	4.8	PPT30yr (100.37, 0.17) **JulyMaxT** (1.64, 0.41) Livestock grazing (0.01, NaN) Insect (1.01, 0.00) Water ponding (1.10, 0.01) **Lichen** (1.90, 0.26)
Black Sage	0.753	5	11.9	Armor (2.85, 0.04) State (2.50, NaN) **Latitude** (0.32, 1.05) **JulyMaxT** (1.70, 0.11) Wildlife (1.10, 0.002)
Bluebunch Wheatgrass	0.706	4	11	State (16.8, 0.14) Soil Depth (3.15, 0.16) **PPT30yr** (40.51, 0.47) **MinVPD** (0.57, 0.31)
Cheatgrass	0.260	3	53.9	State (2.50, 0.00) **JanMinT** (1.76, 0.24) Roads gravel (0.10, 0.00)
Cheatgrass Weedy Forbs	0.417	4	7.8	**Clay** (9.00, 0.30) State (17.50, 0.25) **JanMinT** (0.98, 0.66) Lichen (1.10, 0.002)
Crested Wheatgrass	0.517	3	3.4	**Clay** (19.5, 0.35) **Longitude** (0.81, 2.62) Wildfire (0.10, 0.00)
Field Brome—Little Bluestem	0.09	2	10.8	**Silt** (6.00, 0.35) **Elevation** (104.35, 1.34)
Juniper	0.574	4	6.0	**Armor** (3.50, 0.21) **Latitude** (0.64, 0.28) JanDewP (0.72, 0.19) Water (1.10, 0.03)
Lesser Spikemoss	0.520	4	3.6	Sand (41.25, 0.05) **County** (65.00, 0.75) **JulyMaxT** (0.20, 3.54) Lichen (10.50, 0.12)
Little Sage	0.835	3	5.4	**County** (3.50, 0.98) PPT30yr (123.51, 0.20) **Elevation** (164.85, 0.35)
Mountain Big Sage	0.680	3	8.0	Sand (9.25, 0.13) **MinVPD** (0.75, 0.32) **JanMinT** (1.64, 0.40)
Needle and Thread—Threadleaf Sedge	0.306	8	5.8	**State** (36.00, 0.00) **Longitude** (2.20, 0.14)
Sandberg's Bluegrass	0.496	4	10.5	**JanMinT** (0.78, 1.64) Foot traffic (0.05, 0.00) Wildfire (0.10, 0.00) LC (0.25, NaN)
Shadscale Lichen	0.819	4	3.2	**Silt** (33.00, 0.11) **Longitude** (3.32, 0.36) Non-rodent (0.10, 0.00) Roads dirt (0.10, 0.00)
Western Wheatgrass	0.113	2	3.4	**Latitude** (1.07, 0.19) **Longitude** (0.26, 1.19)
Wyoming Big Sagebrush	0.499	4	27.2	State (7.50, 0.00) **County** (5.10, 0.76) **Latitude** (0.53, 0.69) Livestock (0.10, 0.00)

The best two variables in explaining cover as judged by sensitivity are in bold. xR^2, cross validated R^2; V, number of predictors in the model; N, Average neighborhood size. Directions of relationships at the regional scale can be visualized in ***Figure 2****. Plot locations for each plant community are shown in* ***Supplementary Figure 1****. Predictors related to the abiotic environment and disturbance are described in* ***Supplementary Table 1****. Lichen refers to lichen cover.*

community and shadscale—lichen community (**Figure 1**, **Supplementary Table 2**). Both communities inherently have low cover of herbaceous plants but for opposing reasons. Sagebrush can have large canopies, leaving little exposed interspace for herbaceous species (Ellsworth et al., 2016). However, some mosses prefer filtered light, and are often favored by these microclimates (Proctor and Smirnoff, 2000; Durham et al., 2018). Due to dry, salty conditions, shadscale communities are generally harsh sites requiring more saline-tolerant vascular plants and less productive sites, which make them more amenable to biocrusts (West and Young, 2000). In both scenarios, biocrusts are filling a void that is not easily filled by vascular plants.

The factors that drive the abundance of the lichen and moss components of biocrusts varied by plant community. The Wyoming big sagebrush community demonstrated some of the highest maximum cover values of the lichen component outside of the shadscale-lichen community (nearly 45%), even though average cover values were low (**Figure 1**). In this community, there is a need to manage for cover of biocrusts and particularly the lichen component given historic overgrazing and the association between lichens and reduced cover of cheatgrass (Condon and Pyke, 2018a). The Sandberg's bluegrass community demonstrated relatively low cover values of lichens and moderately high cover values of mosses (**Figure 1**) corroborating descriptions of this community type and biocrust associations in the northern Great Basin (Rosentreter, 1986). Additionally, Sandberg's bluegrass is commonly seeded in restoration projects (Knutson et al., 2014). It is possible that tillage from seeding efforts led to reductions in cover of lichens as has been seen in sagebrush steppe habitats in Montana (Durham et al., 2018). We were unable to reliably model lichen cover in the cheatgrass-weedy forb community because lichens were only recorded in two of these plots (**Figure 1**, **Table 1**). Weedy forbs such as Russian thistle are not very competitive and are characteristic of sites that have recently been heavily disturbed (West and Young, 2000). The high likelihood of recent heavy disturbance in this community may explain the low cover of lichens because lichens are generally sensitive to disturbance (Ponzetti and McCune, 2001; Ponzetti et al., 2007; Condon and Pyke, 2018b). The importance of location-related predictors in the many of plant community specific models of lichen and moss cover suggest that there are environmental or disturbance related predictors that have been missed. Location related predictors may also relate to potentially differing abilities of crews to detect lichens and mosses since crews are often contracted to cover specific areas.

In rangelands of Argentina, Australia, Mexico, Portugal, and Inner Mongolia, biological soil crusts have been evaluated in the relation to grazing practices along gradients or a series of time since disturbance (Liu et al., 2009; Gómez et al., 2012; Concostrina-Zubiri et al., 2014, 2016; Eldridge et al., 2016). Disturbance factors in the AIM dataset are noted as present or absent, possibly masking differences in cover that would be apparent if disturbances were quantified along a continuum. Studies on the effects of grazing have been conducted on biocrusts in portions of the western US but not across the region (Anderson et al., 1982; Ponzetti and McCune, 2001; Condon and Pyke, 2018a,b; Duniway et al., 2018; Condon et al., 2019). Examining the abundance of biocrusts in conjunction with potential stressors could lead to the erroneous conclusion that biocrusts are absent or only present

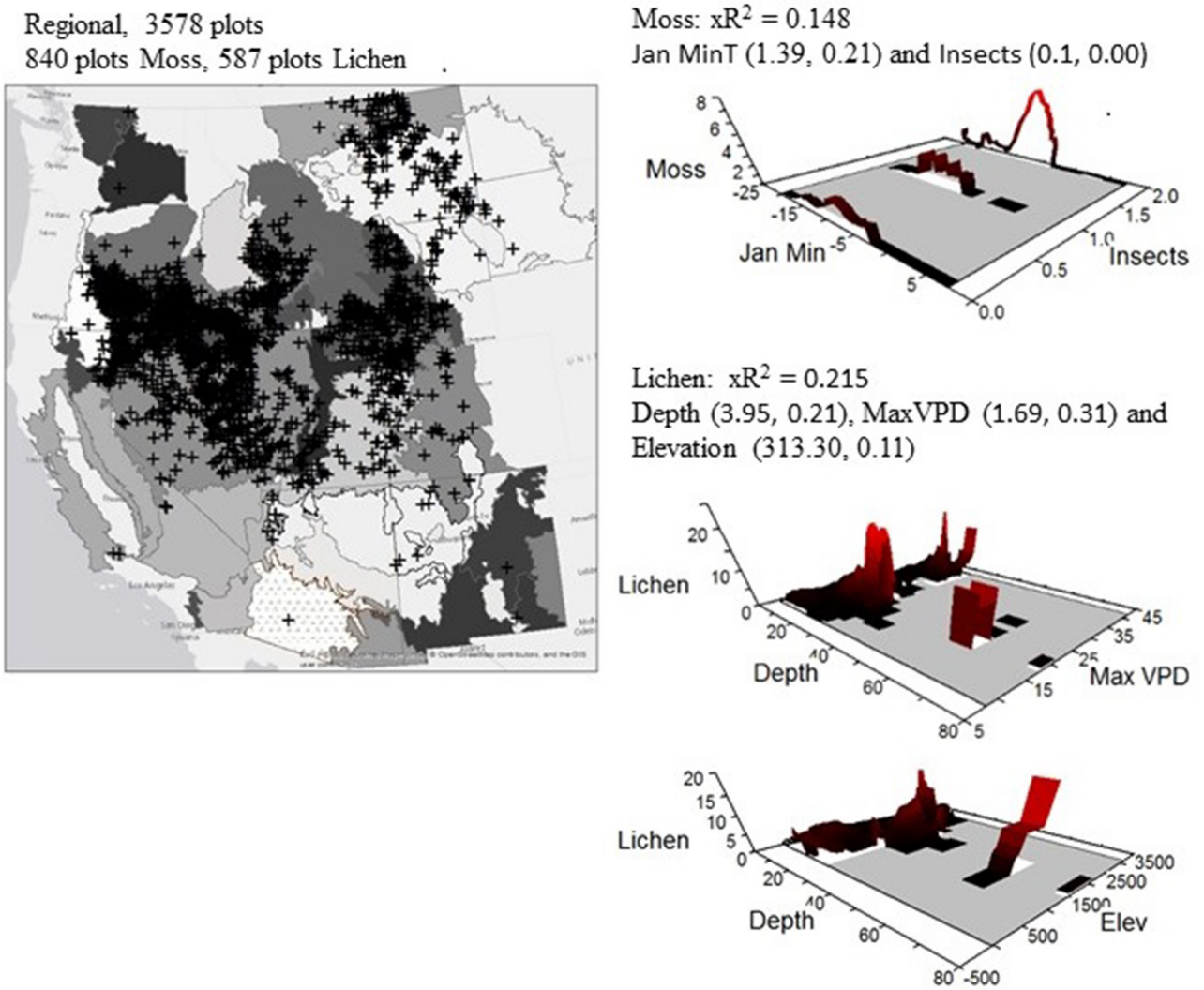

FIGURE 2 | The geographical distribution of the total number of plots classified in each identified plant community and the number of plots that were recorded as having mosses and lichens. The background is the EPA Level 3 ecoregions. Adjacent to the map are NPMR models that of the best regional predictors of lichen cover: maximum vapor pressure deficit (Max VPD), soil depth (Depth) and Elevation and for moss cover: January minimum temperature (JanMinT) and Insects. For each model the cross-validated R^2 (xR^2), tolerance, and sensitivity are reported.

at low levels, when in fact they are being examined under the stressors that keep them as low cover. Under these conditions, it is difficult to separate the effects of current from historic lands uses such as historic overgrazing, abandoned cropland, invasive species, forage seeding, recreation, and changing fire frequencies leading to reductions in cover that are still apparent (Morris and Rowe, 2014; Condon and Pyke, 2018b).

Recent work on biocrusts highlights their potential sensitivities to climate change. It is anticipated that warmer climates will result in the mortality of the lichen and moss components of biocrusts and shift communities to more early successional components such as cyanobacteria (Escolar et al., 2012; Ferrenberg et al., 2015; Maestre et al., 2015). These changes in composition are likely to have cascading effects on global nitrogen and carbon cycles given the trend of later successional biocrusts, including lichens and mosses, to fix more carbon and nitrogen (Houseman et al., 2006; Barger et al., 2013). However, we observed an association between the abundance of lichens and increased aridity (maximum vapor pressure deficit and July maximum temperatures). Our finding that the abundance of the moss component of biocrusts is largely driven by minimum temperatures in January, corroborate this potential trend across the western US. It is well-documented that short, precipitation events in warmer months leads to chlorosis and eventual declines in moss cover (Barker et al., 2005; Coe et al., 2012; Reed et al., 2012; Condon and Pyke, 2016) and that ecotypes that are accustomed to summer monsoons are not associated with stress tolerance (Doherty et al., 2017). The effects of climate change on biocrusts may be self-perpetuating given the ability of some biocrust organisms to alter albedo and surface energy (Couradeau et al., 2016; Rutherford et al., 2017).

Higher cover of biocrust organisms may have been detected if a different sampling method had been used because line-point intercept (LPI) is not the best method for detecting organisms with low cover. A greater number of sampling points are needed to quantify species with low cover (Bonham, 2013). Our data likely underestimates the occurrence of these organisms both due to the number of points used with LPI and because there is a tendency for observers misidentify mosses and lichens that are not obvious and to call them soil or litter. Although we anticipate that some observers missed or misidentified some components of biocrusts, our work demonstrates that biocrusts are found throughout the western US.

The contributions of biocrusts to ecosystem functions need to be further explored both regionally and within plant communities where the roles and composition of biocrust components are likely to differ. Corroborating Belnap et al. (2001), mosses make up a greater proportion of biocrust cover in the more northern part of the region, which aligns with the plant communities that fall within the cold deserts of the Snake River Plain, Northern and Central Basin and Range (**Supplementary Figure 1**). Although mosses have been shown to put on substantial increases in cover every winter in the more northern part of the region (Condon and Pyke, 2016), this trend may not hold in sites that fall outside of the same climatic regimes. Biological soil crusts affect site hydrology with greater infiltration being observed on biocrusts dominated by intact mosses as opposed to disturbed biocrusts or biocrusts dominated by lichens (Brotherson and Rushforth, 1983; Chamizo et al., 2012). Biocrusts may increase runoff in the case of early successional, light cyanobacterial crusts (Faist et al., 2017) but may ultimately reduce runoff, leading to greater net infiltration compared to areas without biocrusts due to the microtopography that they maintain (Warren, 2003). These are critical relationships in arid and semi-arid environments where plant establishment is limited by available water (Bainbridge, 2007). Biological soil crusts are also contributors to the ecosystem function nutrient cycling which is of paramount importance in these semi-arid ecosystems. *Bromus tectorum* L. and other ruderal and weedy plant species benefit from self-perpetuating nutrient cycles, particularly excess nitrogen (Sperry et al., 2006). Scientists continue to explore management actions that will reduce labile soil nutrients in efforts to reduce *B. tectorum* (Jones et al., 2015). The restoration of biocrusts could be considered in these efforts as biocrusts fix carbon (Evans and Lange, 2003) and carbon additions increase the carbon/nitrogen ratio, reducing the amount of nitrogen that is available for uptake by vegetation (Mazzola et al., 2008). Our study demonstrates the potential for biocrusts to be in all plant communities across the western United States justifying their inclusion in restoration and management targets.

CONCLUSIONS

We demonstrate the presence of the lichen and moss components of biocrusts in all plant communities across the semi-arid western US, concluding that the absence of the lichen and moss components of biocrusts is not a result of their being unable to grow in the environmental setting in any of our identified plant communities. Maximum vapor pressure deficits, soil depth and elevation are regionally important for lichen cover, but moss cover is largely driven by minimum temperatures in January suggesting that potential changes in these associated climatic variables are likely to influence the prevalence of the biocrust components.

Throughout the region, plant communities are being related to potential vegetation types or Ecological Site Descriptions (ESDs), based on information about soils and climate. Existing ESDs are currently being used to understand ecosystem dynamics and responses to disturbance allowing for the prediction of changes in dominant plant species following thresholds of disturbance (Stringham et al., 2003; Brown and Bestelmeyer, 2016). Our work provides a foundation for the inclusion of biocrusts in the ESD framework with reported maximum values of moss and lichen cover in each identified plant community. Future examinations of disturbance thresholds with the objective of understanding the recovery of ecosystem processes would be more complete with the inclusion of biocrusts.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the Bureau of Land Management https://landscape.blm.gov/geoportal/catalog/AIM/AIM.page.

AUTHOR CONTRIBUTIONS

LC and DP conceived the study and wrote the paper. LC analyzed the data.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00449/full#supplementary-material

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Bacterial Communities of Mojave Desert Biological Soil Crusts Are Shaped by Dominant Photoautotrophs and the Presence of Hypolithic Niches

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Biocrust communities are often heterogeneous and affected by small-scale environmental features, including both physical and biotic factors. The presence of moss in biocrusts, for example, is likely to create a set of physical conditions distinct from those found in microbial biocrusts that lack mosses. Similarly, hypolithic organisms living under and on the belowground surface of translucent and opaque stones (typically quartz) experience a distinct environment relative to surrounding soil surface organisms. To understand the impact of these factors on biocrust bacterial community composition, we performed 16S rRNA sequencing from surface and hypolithic biocrusts with and without the common biocrust moss, *Syntrichia caninervis*. While alpha diversity indices did not differ significantly between any of the biocrust habitats sampled, we did observe differences in overall community composition. Cyanobacterial abundance and diversity decreased in the presence of moss and in surface samples compared to hypolithic biocrusts, while Proteobacteria showed the opposite pattern. Acidobacteria were significantly more abundant in hypolithic niches, and Patescibacteria were found to be restricted to moss-dominated surface biocrusts. Notably, bacterial community composition was found to shift significantly between surface and hypolithic microbial biocrusts (Adonis, $R^2 = 0.122$, $p = 0.002$) and between surface moss and microbial biocrusts ($R^2 = 0.107$, $p = 0.002$). These findings support the idea that even at small spatial scales (e.g., within <10 cm), desert biocrust bacterial community composition varies based on the habitat structure and cohabitants.

Keywords: biological soil crust, bacterial community, hypolithic, *Syntrichia caninervis*, 16S rRNA

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INTRODUCTION

Competitive exclusion and habitat selection theory posit that differential habitat selection and niche partitioning may permit organisms of similar phenotypes to coexist (Darwin, 1859; Gause, 1934; Hutchinson, 1959; Rosenzweig, 1981). In soil microbial communities, diversity may be maintained through the spatiotemporal partitioning of habitats and associated resources (Ettema and Wardle, 2002; Lee et al., 2016). As a driver of community diversity, niche partitioning is an important

process to understand in the context of global change, as higher diversity is expected to enhance the resilience of communities in the presence of disturbance (MacArthur, 1955; Giller et al., 1997; Girvan et al., 2005).

The importance of niche partitioning coupled with facilitative interactions is particularly pronounced for soil biological crust (biocrust) communities, which comprise diverse heterotrophic microorganisms that are dependent on the resources generated by photoautotrophs (Baran et al., 2015; Maier et al., 2018; Couradeau et al., 2019). Biocrust composition varies with physical factors as well as age and successional stage, but these communities may include fungi, lichens, mosses, green algae, diatoms, and archaea in addition to diverse bacteria (Belnap and Lange, 2003). Biocrusts predominate in dryland ecosystems, where they represent an estimated 40–50% of surface cover (Elbert et al., 2012; Garcia-Pichel et al., 2013) and are responsible for significant ecosystem services, including carbon cycling and nitrogen fixation (Belnap and Lange, 2003; Elbert et al., 2012).

Biocrusts are frequently considered successional communities, and successional stages are classified by the dominant photoautotroph, with early-stage biocrusts characterized by colonizing cyanobacteria (e.g., *Microcoleus vaginatus*) and mid to later stages dominated by lichens and mosses, respectively (Weber et al., 2016; Mogul et al., 2017; Maier et al., 2018). However, independent of succession, the presence or absence of particular biocrust photoautotrophs and their bacterial cohabitants can also be controlled by abiotic features of the local environment, such as moisture availability and substrate stability (Kidron et al., 2000, 2009). At meso- to micro-scales, the development of biocrusts and the establishment of their constituent photoautotrophs is dependent on edaphic factors (e.g., soil pH, nutrients, and texture) and local habitat features such as slope, orientation, and shading from vascular plants (Bowker et al., 2016; Mogul et al., 2017; Durham et al., 2018). At this small local scale, biocrust organisms can be further partitioned into hypolithic habitats, which occur under the ventral surfaces of translucent and opaque stones (typically quartz) that are embedded in the soil surface (Chan et al., 2012). Hypolithic habitats are commonly found in drylands, although they can occur anywhere suitable substrate is available (Pointing, 2016). In addition to reduced UV radiation and buffering from extreme thermal fluctuations, organisms in these niche habitats experience enhanced water availability relative to that in surrounding surface soils due lower evaporation, higher relative humidity, and capture of water via fog condensation (Pointing, 2016). Nonetheless, dryland hypolithic habitats are still colonized by poikilohydric organisms that must withstand extended periods without water (Pointing and Belnap, 2012).

Although not always explicitly recognized as biocrusts, the microbial communities associated with hypolithic habitats have been well-characterized globally (Chan et al., 2012; Makhalanyane et al., 2013; Stomeo et al., 2013; Le et al., 2016; Vikram et al., 2016; Lacap-Bugler et al., 2017; Van Goethem et al., 2017). Cyanobacteria are the most common and abundant organisms in hypolithic biocrust communities (Chan et al., 2012; Pointing, 2016), with taxa from the genus *Phormidium* dominant in Antarctic and Tibetan hypoliths and *Chroococcidiopsis* most abundant in hot and cold deserts (Lacap-Bugler et al., 2017). Comparisons of microbial community composition in hypolithic and adjacent surface soil habitats have typically supported the idea that hypolithic niches harbor unique assemblages of bacteria that are distinct from, and not a filtered subset of, surface soil communities (Khan et al., 2011; Stomeo et al., 2013; but see also Makhalanyane et al., 2013; Le et al., 2016).

Although less common, hypolithic biocrusts sometimes include mosses (Cockell and Stokes, 2006; Cowan et al., 2010, 2011; Chan et al., 2012; de los Ríos et al., 2014). In surface biocrusts, the presence of mosses is assumed to modulate microbial community composition (Moquin et al., 2012; Antoninka et al., 2015; Xiao and Veste, 2017), presumably because their additional biomass modifies the physical and chemical environment experienced by the biocrust microbial community (Antoninka et al., 2015; Xiao et al., 2016). Moss-dominated surface biocrusts are also capable of buffering important ecosystem functions such as nitrogen, carbon, and phosphorous cycling from the detrimental effects of increased aridity in deserts and drylands (Delgado-Baquerizo et al., 2016). Most work characterizing moss bacterial community composition has compared moss biocrusts to nearby soils that do not support biocrusts (Moquin et al., 2012; Delgado-Baquerizo et al., 2016; Xiao and Veste, 2017). However, studies directly comparing biocrusts with different dominant photoautotroph have found that moss-dominated biocrusts harbor a microbial community taxonomically and functionally distinct from that of cyanobacteria-dominated biocrusts (Kidron et al., 2010; Maier et al., 2018). Similarly, comparisons of bacterial community composition from hypolithic and adjacent surface niches thus far have focused on extreme environments (e.g., the Antarctic, Namib, and Atacama Deserts) that do not support well-developed surface biocrusts (Makhalanyane et al., 2013; Stomeo et al., 2013; Le et al., 2016; Vikram et al., 2016). Thus, the role that hypolithic niches interspersed within broader surface biocrusts might play in promoting local-scale beta diversity remains relatively unexplored.

Because of the potential contributions of habitat partitioning and facilitative interactions to the overall diversity and associated ecological resilience of biocrust microbial communities, in this study we set out to characterize the relative impacts of hypolithic niche and dominant photoautotroph on bacterial community composition. We sampled biocrusts with either cyanobacteria or moss as the dominant photoautotroph from both surface and hypolithic microhabitats within a small (8 m^2) area in the Mojave Desert, and used 16s rDNA sequencing to test the hypothesis that both the presence of moss and hypolithic microhabitat would have significant impacts on the composition of bacterial communities associated with each type of biocrust.

METHODS

Field Site and Sample Collection

Soil and biocrust samples were collected on March 25, 2018 from the Sheep Creek Wash near Wrightwood, CA. The Sheep Creek Wash site is located at the northern base of the San Gabriel

Mountains (34°22′ 33.85″N, 117°36′34, 59 W) and the western edge of the Mojave Desert at an elevation of 1,800 m (Baughman et al., 2017). This site was chosen based on the presence of both surface and hypolithic *Syntrichia caninervis* biocrusts within the same restricted (∼8 m^2) area. *Syntrichia caninervis* was detected based on characteristics such as hair points on the apices of leaves, leaf morphology, and colony pigmentation. Biocrusts with cyanobacteria as the dominant photoautotroph were identified based on their adherent texture and darker pigmentation relative to the underlying sand substrate. To differentiate the description of crust type based on dominant photoautotroph (cyanobacteria-dominated vs. moss-dominated) from the description of biocrust bacterial community composition (which will likely always include cyanobacteria in some proportion), for the remainder of this report we use the term "microbial biocrust" to refer to biocrusts with cyanobacteria as the dominant photoautotroph. Seven replicate samples were collected for each of the following microsite types: surface moss biocrust; surface microbial biocrust; hypolithic moss biocrust; hypolithic microbial biocrust; sub-biocrust soil (for all previous sample types); and non-biocrust surface soil. Abiotic features of the habitat such as slope, aspect, and sun exposure can influence the establishment of dominant photoautotrophs such as mosses and cyanobacteria (Kidron et al., 2000, 2009, 2010), and thus might also directly select for particular bacterial assemblages. Because we aimed to focus our study on the impact of biocrust mosses and hypolithic microsites on bacterial community composition, we designed our collection strategy to minimize variation in local abiotic factors across the biocrusts sampled. All samples were collected from the same shelf within the Sheep Creek Wash, which was ∼2 × 4 m and contained a mosaic of intermixed microbial (cyanobacteria-dominated) and moss biocrusts with quartz and other stones sporadically embedded within. The sampling area experienced uniform shading from two trees (*Juniperus occidentalis*) to the east and southeast of the shelf. While there was some variation in the microtopography across the sampling area (1–3% slope change from the center of the shelf to the lower edge), both surface and hypolithic moss and microbial biocrusts were distributed throughout the entire area, and replicate samples of each crust type were collected from both the interior and outer edges of the sampling area to capture the range of slope variation. For each hypolithic sample we collected, we also collected a sample of the surface biocrust (moss or microbial) directly abutting the hypolith quartz rock (**Figure 1**). To limit our comparisons to moss and microbial photoautotrophs, we avoided collecting biocrusts with lichens present. A sterile spatula (surface sterilized with 70% isopropyl alcohol between samples) was used for collection of 5–10 g soils and biocrusts, and each sample was placed individually into a sterile Nasco Whirl Pak bag (Fort Atkinson, WI). For hypolithic samples, quartz rocks (often with visible adhered microbial biomass) were collected along with soil and biocrust. All samples were stored on ice during field collection and transport back to the lab, where they were stored at −20°C until DNA extraction.

Sample Preparation and DNA Extraction

Quartz samples were crushed with a UV sterilized hammer to obtain biological matter adhered to the rock samples. Smaller rocks were scraped using a sterile scalpel to gather biological materials for DNA extraction. For samples containing moss biocrusts, ca 5 stems of moss were first submerged for several seconds (using sterile forceps) in the buffer used during cell disruption step of the DNA extraction protocol, to remove some of the adhered soil and biocrust material for subsequent DNA extractions. Care was taken to remove all traces of moss after submersion. The prepared samples then underwent DNA extraction using a QIAGEN DNeasy PowerSoil Kit (Qiagen, Germantown, MD) according to the manufacturer's instructions (with the addition of the moss-washing step noted above). Quantification readings were taken immediately after DNA extraction using a Qubit 4 fluorometer (Invitrogen, Carlsbad, CA).

16s rRNA Amplification and Sequencing

The V3 and V4 hypervariable region within the 16S rRNA cistron was amplified using primer sequences 341F and 805R with Illumina sequencing adaptors (Herlemann et al., 2011; Mizrahi-Man et al., 2013). All PCR reactions contained 12.5 μl of Apex HotStart Master Mix, 8.5 μl of sterilized molecular biology-grade water, 1.0 μl of 10 mM forward and reverse primers, and 2.0 μl of DNA template. Each PCR reaction was performed using the following cycling parameters: initial denaturation of 95°C for 3 min, denaturation of 95°C for 30 s, annealing (generating 25 cycles) of 55°C for 30 s, extension of 72°C for 30 s, final extension of 72°C for 30 s. Positive (1 mg/μl bacterial community DNA, ZymoBIOMICS Microbial Community DNA Standard, Zymo Inc.) and negative controls were included in these PCR runs.

To visually confirm successful amplification, all PCR products were run in 0.5X TBE buffer on a 2% agarose gels. Each gel contained 6 μl DNA Ladder II 1 (1 kb) and 6 μl of each DNA sample, followed by staining with ethidium bromide. Upon confirmation of the target ∼500 bp amplicons, PCR products were sent to ChunLab (ChunLab, Inc., Seoul, South Korea) for sequencing on the Illumina MiSeq platform. Because the negative controls failed to yield any amplification products, only positive controls were sent for sequencing.

Sequence Assembly and Analysis

Demultiplexed paired end MiSeq reads for 40 samples were trimmed, filtered, denoised, and merged using the DADA2 (Callahan et al., 2016) plugin in QIIME2 (Bolyen et al., 2018). Sequences were assigned to taxa using the scikit-learn 0.20.2 native Bayes classifier (Pedregosa et al., 2011) trained on the Silva v.132 reference database (Quast et al., 2013; Yilmaz et al., 2014). The q2-taxa plugin in QIIME2 was used to filter mitochondrial and chloroplast sequences from the dataset prior to downstream analyses.

For the calculation of phylogenetic diversity metrics (Faith's, unweighted UniFrac), positive control samples were removed and a rooted phylogeny was generated from the sequences using

FIGURE 1 | Habitat photo (Left), showing a major portion of the collection area with locations of two hypoliths harboring moss biocrusts indicated [**A,B**—corresponding to the photos in **(A,B)** to the right of the figure]. Representative moss **(C)** and microbial **(D)** biocrusts are also indicated. Inset **(A)**: moss hypolith showing mosses in their hydrated state; the surface biocrust adjacent to this hypolith is microbial, toward the upper left of the panel. Inset **(B)**: moss hypolith in desiccated state, with moss biocrusts on the soil surface adjacent to the hypolith.

the QIIME2 q2-phylogeny plugin's align-to-tree-mafft-fasttree pipeline. Standard alpha (Shannon's, evenness, Faith's) and beta (Bray-Curtis, unweighted and weighted UniFrac) diversity metrics were calculated with the QIIME2 q2-diversity plugin using a data set rarefied to 9,000 OTUs per sample. This sampling depth was selected based on rarefaction curves for observed OTUs generated for each sample type at sampling depths ranging from 1 to 10,000. Alpha diversity measures were explored for associations with habitat metadata (crust location and biocrust composition) to test for significant differences in community diversity between different biocrust microhabitats.

Sample metadata, the QIIME2 feature abundance table (containing the frequencies of each OTU), and the QIIME2 taxonomy table (containing the taxonomic assignments of each OTU) were imported into the Calypso online software suite for microbiome analysis (Zakrzewski et al., 2017). Prior to analyses, the two positive control samples and the non-crust (soil only) samples were excluded from the data set. Rare OTUs with $<0.01\%$ relative abundance across all samples were also removed. Abundance data for the 3,418 taxa present in the remaining 28 samples were normalized through cumulative-sum scaling and $\log_2$ transformed.

An Adonis PERMANOVA of Bray-Curtis distances was used to test for significant compositional differences between moss and microbial biocrusts in surface and hypolithic niches. To compare overall community composition across biocrust sample types, we also performed non-metric multidimensional scaling using Bray-Curtis distances. Multivariate ordinations were performed first for all four biocrust sample types combined. To better resolve the individual influences moss and hypolithic niche on community composition, we then performed Adonis and NMDS for pairwise comparisons of samples grouped by biocrust composition (with or without moss) and by location (surface or hypolithic). Univariate analyses (one-way ANOVA) were implemented to determine which taxa (phyla, genera) displayed significant differential abundance between sample types.

To generate taxonomic community profiles scaled to relative abundance within sample types, the original unfiltered, non-normalized data were imported into phyloseq (McMurdie and Holmes, 2013), where the same filters were applied as described above. Samples from identical biocrust types were then merged and OTU abundance data were normalized to relative abundance within sample type prior to generating taxonomic community profiles.

RESULTS

Sequence Assembly

After filtering, denoising, merging, and removal of chimeric sequences, sequencing depth of the field-collected samples ranged from 4,184 to 20,010 (mean = 11,054).

The two mock microbial community positive control samples were consistent with each other in composition and did not share any sequences in common with the rest of the samples. Together they contained 22 OTUs, all of which represented sequences highly similar or identical to those from the eight species present in the ZymoBIOMICS standard. In total, 18,490 features were

recovered in the assembly, with the depth of coverage per feature ranging from 1 to 2,284 (mean = 23).

Diversity Measures

Shannon indices did not indicate overall significant differences in alpha diversity among any of the sampled biocrust or soil habitats (Kruskal-Wallis $p = 0.245$), and no differences in Shannon diversity were found in pairwise comparisons of samples filtered by biocrust composition (moss vs. microbial crust samples in hypolithic sites, $p = 0.223$; in surface sites, $p = 0.631$) or by sampling location (hypolithic vs. surface moss crusts, $p = 0.584$; hypolithic vs. surface microbial crusts, $p = 0.199$). Similar to Shannon diversity measures, Faith's Phylogenetic Diversity (PD) did not vary significantly in any of these pairwise comparisons. Evenness was not found to vary significantly among sample types overall (group Kruskal-Wallis $p = 0.311$), although a pairwise comparison of microbial crusts did indicate significantly higher evenness of bacterial communities in hypolithic compared to surface sites ($p = 0.045$).

Multivariate Analyses of Community Composition

Adonis PERMANOVA of Bray-Curtis distances indicated significant overall differences in composition between biocrust sample types ($R^2 = 0.142$, $p = 0.0003$). Further partitioning of the sample data to pairwise comparisons within one biocrust type or one biocrust niche revealed these patterns to be driven largely by compositional differences between surface moss and microbial biocrusts ($R^2 = 0.092$, $p = 0.038$) and between surface and hypolithic biocrusts without moss ($R^2 = 0.115$, $p = 0.002$). Adonis did not distinguish samples from surface and hypolithic moss biocrusts ($R^2 = 0.88$, $p = 0.08$), or hypolithic moss and microbial biocrusts ($R^2 = 0.83$, $p = 0.19$).

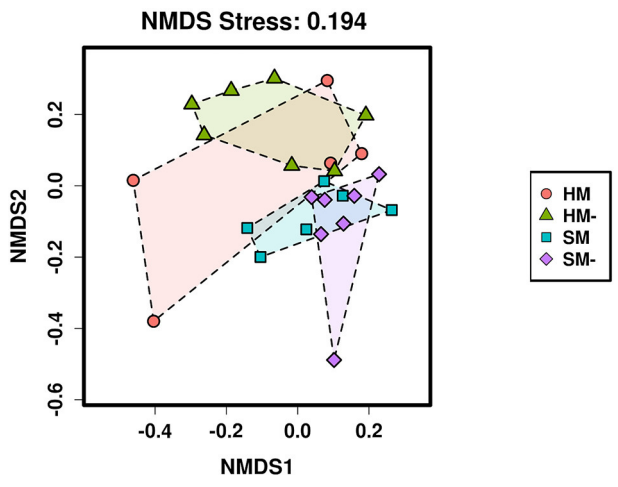

FIGURE 2 | Plot of the non-metric multidimensional scaling ordination of Bray-Curtis distances for the four sampled biocrust habitats. HM, hypolithic moss biocrust; HM-, hypolithic microbial biocrust; SM, surface moss biocrust; SM-, surface microbial biocrust.

Non-metric multidimensional scaling (NMDS) suggested compositional differences between at least some of the four biocrust habitats we sampled (**Figure 2**). When the samples were partitioned to compare composition within each type of biocrust by habitat and vice-versa, NMDS ordinations were consistent with the Adonis results, visually distinguishing communities from hypolithic and surface biocrusts without moss and from moss and microbial surface biocrusts (**Figure 3**).

Community Profiles and Differential Taxon Abundance in Sampled Biocrusts

Community profiles reflected shifts in the relative abundance of all phyla present in the sampled biocrust habitats, showing a trend toward increased cyanobacterial abundance in biocrusts without moss present (**Figure 4**). Relative abundance profiles of cyanobacterial genera across the four biocrust communities and adjacent non-biocrust soils revealed increased diversity of cyanobacterial taxa in hypolithic biocrusts compared to surface biocrusts and bare soils (**Figure 5**). ANOVA comparisons of phylum abundance indicated significant differences in the abundance of Chloroflexi ($p = 0.00056$), Deinococcus-Thermus ($p = 0.0024$), Acidobacteria (0.015), and Elusimicrobia ($p = 0.021$) within the 28 biocrust-associated samples (**Figure 6**). When samples were restricted to one sampling location (surface or hypolithic), surface environments ($n = 16$) showed significant differential abundance of Patescibacteria ($p = 0.032$), Chloroflexi ($p = 0.038$), and Planctomycetes ($p = 0.042$), all of which were more abundant in moss-dominated surface biocrusts. In hypolithic environments ($n = 16$), Elusimicrobia ($p = 0.022$), and Deinococcus-Thermus ($p = 0.04$) were differentially abundant with significantly higher prevalence in biocrusts without moss. Grouping samples by dominant photoautotroph (moss or microbial) in the ANOVA revealed that moss-dominated biocrusts ($n = 16$) harbored significantly more Acidobacteria ($p = 0.015$) in hypolithic niches, while Planctomycetes ($p = 0.021$) and Patescibacteria ($p = 0.027$), were more abundant in surface moss biocrusts. In microbial biocrusts ($n = 16$), Deinococcus-Thermus ($p = 0.00073$), Chloroflexi ($p = 0.00087$), Bacteriodetes ($p = 0.34$), and Acidobacteria ($p = 0.41$) all showed significant differential abundance, with all but Bacteriodetes having higher abundance in hypolithic compared to surface crusts lacking moss.

To provide more resolution regarding the taxa associated with significant shifts in community composition, ANOVA tests for differential abundance were run at the generic level for both surface moss and microbial biocrusts, and for microbial biocrusts in surface and hypolithic niches. In surface biocrusts, the presence of moss significantly increased the abundance of *Tepidisphaera*, *Hymenobacter*, *Rhizobacter*, *Sphingomonas*, *Spriosoma*, *Bryobacter*, and *Pseudonocardia* relative to biocrusts lacking moss, which supported significantly more *Aquabacterium*, *Oligoflexus*, *Caenimonas*, *Sporocytophaga*, *Rhodocytophaga*, and *Arctibacter* (**Figure 7**). In microbial biocrusts, hypolithic samples contained a significantly higher abundance of *Truepera*, *Trichocoleus*-SAG-26.92, *Solirubrobacter*, *Rubrobacter*, PMMR1, *Phormidium*-SAG-37.90, *Parviterribacter*, *Flavitalea*,

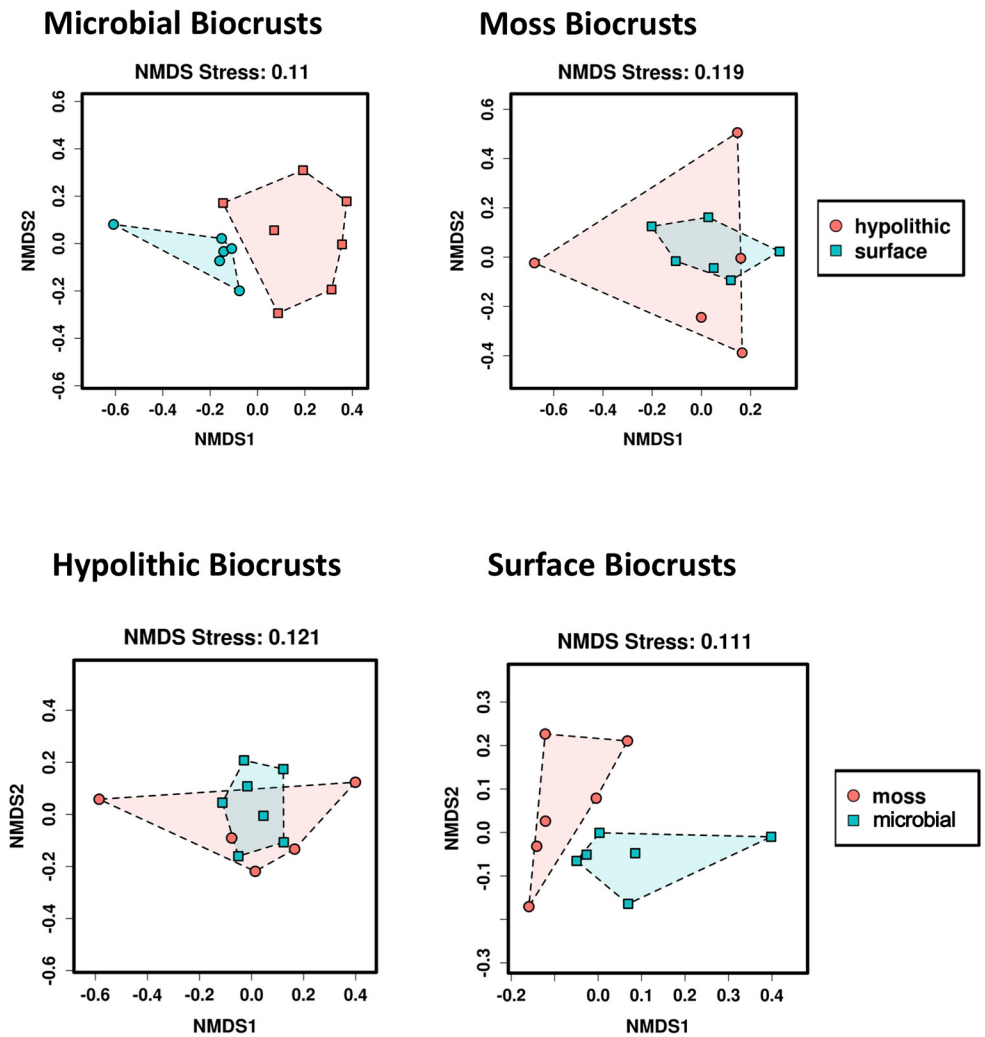

FIGURE 3 | Plots for pairwise non-metric multidimensional scaling (NMDS) ordinations of Bray-Curtis distances. Upper panels show comparisons of microbial **(Left)** and moss **(Right)** biocrusts from hypolithic and surface niches; lower panels show comparisons of microbial and moss biocrusts from hypolithic **(Left)** and surface **(Right)** niches.

Fimbriiglobus, *Conexibacter*, *Bryobacter*, and *Angustibacter* (**Figure 8**). Surface microbial biocrusts, in contrast, contained significantly higher abundance of *Taibaiella*, *Sporocytophaga*, *Silvanigrella*, *Segetibacter*, *Pseudoxanthomonas*, *Peredibacter*, *Flavobacterium*, *Flavisolibacter*, *Fibrella*, *Cellvibrio*, *Blastococcus,* and *Aquabacterium* (**Figure 8**).

DISCUSSION

Biocrusts and the essential ecosystem services that they provide are threatened by anthropogenic disturbances, including physical damage (Weber et al., 2016; Durham et al., 2018) and climate change (Belnap et al., 2004; Delgado-Baquerizo et al., 2016). Since biocrust resistance and resilience to such disturbances is likely to be influenced by community composition (Girvan et al., 2005), understanding factors that may impact local biocrust microbial diversity is critical for predicting responses to disturbance and designing effective restoration strategies.

The results of this study corroborate previous work noting the influence of dominant photoautotrophs in shaping bacterial community composition in surface biocrusts (Maier et al., 2014, 2018), as well as studies that have found compositional shifts with biocrust development or succession (Mogul et al., 2017; Maier et al., 2018). Consistent with these studies, we found significant differences in the composition of bacterial communities from surface moss- and cyanobacteria-dominated biocrusts (Maier et al., 2018). Contrary to these studies, we did not find significant differences in alpha diversity measures among the four biocrust community types we sampled (Maier et al., 2018). However, our study does suggest that beta diversity increases with the co-occurrence of biocrusts with and without moss as the dominant photoautotroph.

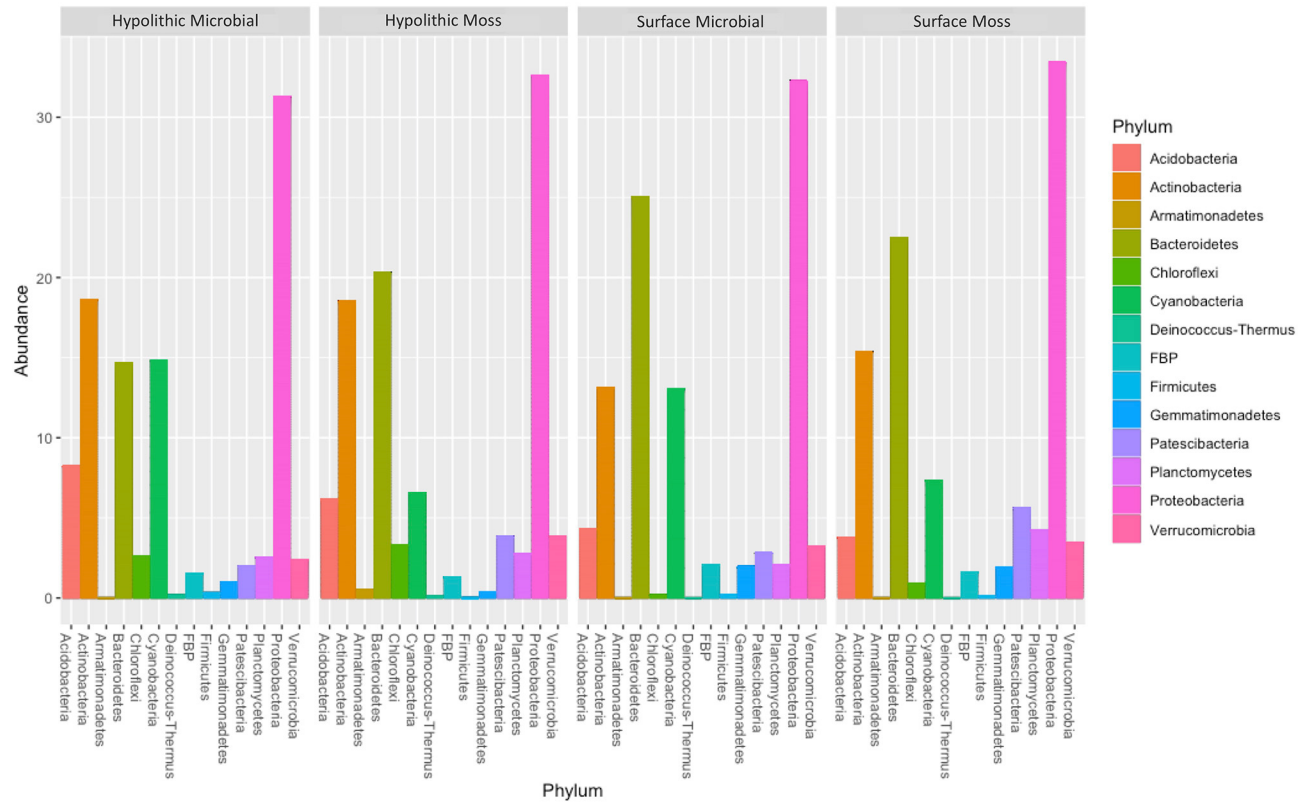

FIGURE 4 | Community profile plots showing the relative abundance of all phyla found in the four sampled biocrust habitats ($N = 7$ for each habitat). OTUs with <0.01% abundance across all samples were removed prior to analysis; remaining abundance data were normalized through cumulative-sum scaling and $\log_2$ transformed.

Unlike surface biocrusts, the communities associated with biocrusts in hypolithic spaces were not significantly impacted by the presence of moss, although we did observe an overall reduction in the abundance of cyanobacteria in hypoliths with moss. These results are consistent with the idea that hypolithic niches exert a strong selective habitat filter, as has been noted in comparisons of cyanobacterial community composition in hypolithic habitats from deserts worldwide (Lacap-Bugler et al., 2017). The unique selective filter imposed by the conditions in hypolithic spaces may attenuate species interactions such as competition, which could explain our observation that OTUs in hypolithic microbial biocrusts displayed significantly higher evenness compared to their surface counterparts.

Comparative work on hypolith bacterial community composition at a regional scale has focused hyperarid deserts that support very little soil biomass beyond hypolithic communities (Cowan et al., 2010; Stomeo et al., 2013; de los Ríos et al., 2014). Thus, comparisons of hypolith and non-hypolith communities in these studies have involved nearby soils without biocrusts, and have concluded that the community in hypolithic environments is primarily filtered from the regional pool of soil microbes (Makhalanyane et al., 2013). The processes influencing hypolith community composition are likely to be more dynamic in regions where both hypoliths and surface biocrusts coexist, and in these systems, hypolithic communities may serve to "seed" surrounding soils with biocrust-forming taxa (Chan et al., 2012).

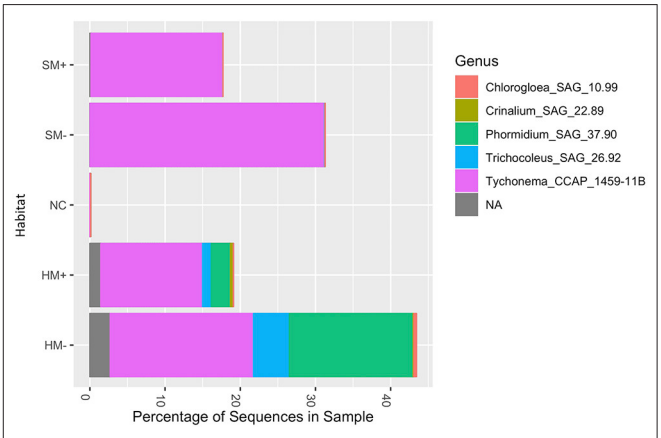

FIGURE 5 | Community profile plots showing the relative abundance of Cyanobacteria genera found the four sampled biocrust habitats and bare soil. OTUs with <0.01% abundance across all samples were removed prior to analysis; remaining abundance data were normalized through cumulative-sum scaling and $\log_2$ transformed. HM, hypolithic moss biocrust; HM-, hypolithic microbial biocrust; SM, surface moss biocrust; SM-, surface microbial biocrust; NC, non-crusted bare soil.

The comparisons reported here provide some evidence supporting this refugial role for hypoliths. Two cyanobacterial taxa were significantly more abundant in hypolithic as opposed to surface microbial biocrusts, *Phormidium* SAG 37.90 ($p = 0.0064$) and *Trichocoleus* SAG 26.92 ($p = 0.0036$), which are associated with temperate freshwater (Marquardt and Palinska, 2007) and hot desert biocrust environments (Lange et al., 1992, as *Microcoleus sociatus*), respectively. These seemingly conflicting habitat associations may reflect the natural disturbance regime in the Sheep Creek Wash where we collected our samples: while the sampling area was located on an elevated, stable shelf within the shallow wash, it is likely that the seasonal flow of water in the wash occasionally covers the shelf. This periodic seasonal disturbance may explain the freshwater habitat associations for many of the taxa highlighted in our differential abundance analyses and community profiles. For example, both moss and microbial biocrusts in surface environments were dominated by a single genus of cyanobacteria, *Tychonema* CCAP 1459-11, an oscillatoroid species typically associated with freshwater environments (Suda et al., 2002). Surface biocrusts without moss are the least physically stable of the four biocrust types we sampled, and thus may be the most vulnerable to occasional disturbance. While periodic flooding may eliminate some taxa from biocrusts on the surface, these taxa may persist in protected hypolithic niches, or in more stable moss biocrusts (see below).

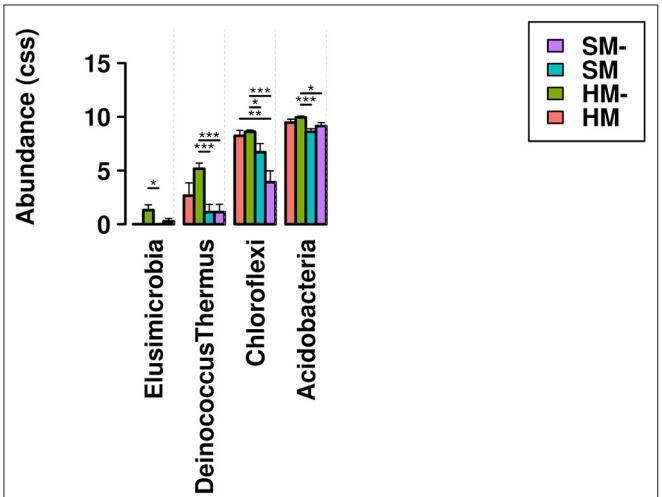

FIGURE 6 | Relative abundance of phyla found to be differentially abundant in an ANOVA of the four sampled biocrust habitats. Bars indicate significant pairwise differences at * ≥ 0.05; ** ≥ 0.01; *** ≥ 0.001. HM, hypolithic moss biocrust; HM-, hypolithic microbial biocrust; SM, surface moss biocrust; SM-, surface microbial biocrust.

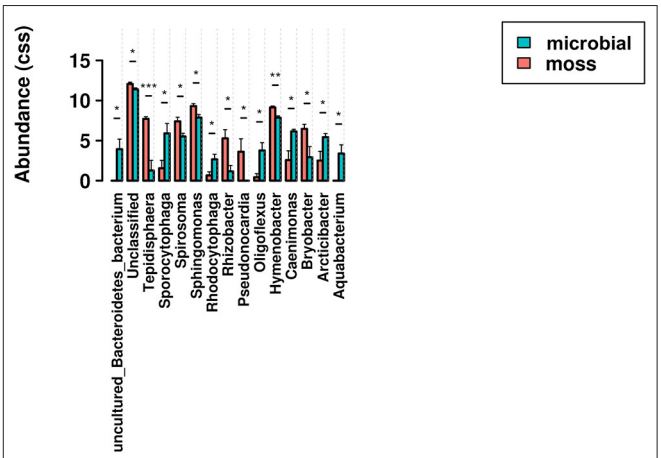

FIGURE 7 | Relative abundance of genera found to be differentially abundant in an ANOVA comparison of surface biocrusts with and without moss. Bars indicate significant pairwise differences at * ≥ 0.05; ** ≥ 0.01; *** ≥ 0.001.

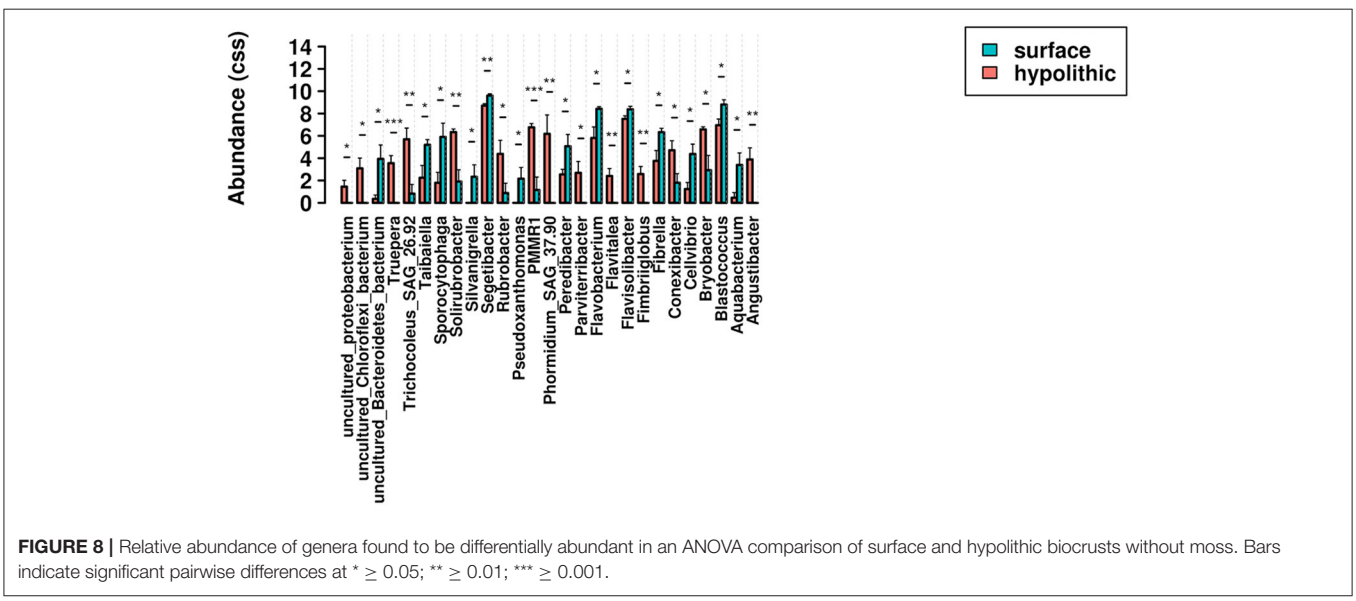

FIGURE 8 | Relative abundance of genera found to be differentially abundant in an ANOVA comparison of surface and hypolithic biocrusts without moss. Bars indicate significant pairwise differences at * ≥ 0.05; ** ≥ 0.01; *** ≥ 0.001.

The two taxa for which we found the most significant increase in abundance from surface to hypolithic microbial biocrusts are both potential extremophiles, *Truepera* ($p = 0.0005$) and *Caulobacteraceae* sp. PMMR1 ($p = 0.0004$). This unexpected association of extremophilic taxa with hypolithic niches, where conditions are expected to be less stressful than on the surface, may reveal a role for hypolithic spaces in providing refugia from occasional disturbances.

In contrast to hypoliths without moss, hypolithic moss biocrust communities were not statistically different from surface moss biocrusts in the Adonis analysis or NMDS ordination. Similar to hypolithic niches, moss biocrusts on the surface may offer some physical protection from low-level disturbances like those described above. This result may also indicate that the presence of moss alters the physical environment in surface biocrusts such that light and moisture conditions experienced by microbes associated with moss biocrusts are similar enough to conditions in hypolithic niches to support partially overlapping bacterial communities.

Recent work on biocrust restoration has emphasized the utility of mosses such as *Syntrichia caninervis* and *S. ruralis* for reestablishing biocrust communities and their ecological function (Antoninka et al., 2015, 2018). Mosses are particularly promising components of biocrust restoration inocula, as they stabilize soils, enhance moisture retention, and facilitate the establishment of other functionally important biocrust microorganisms (Antoninka et al., 2015). Our results corroborate the role of mosses in modulating biocrust bacterial community composition, and expanded metagenomic investigation of the biocrusts investigated here will provide further resolution regarding the potential facilitative role of mosses by characterizing functional variation accompanying shifts in bacterial community composition with dominant photoautotroph. While the composition of communities in hypolithic spaces has previously been considered without direct comparison to nearby surface biocrusts, our results highlight the potential role of hypolithic niches in enhancing bacterial diversity at sites containing established surface biocrusts. If future biocrust restoration projects deploy greenhouse-grown biocrust mosses in an effort to expedite re-establishment of more complex, late-successional communities (Antoninka et al., 2018), such efforts might be enhanced by simultaneous introduction of quartz stones, sporadically embedded in the soil surface within the area under restoration (Chan et al., 2012). Deliberate introduction of such hypolithic spaces could encourage the establishment of taxa that might otherwise be absent, and could potentially provide refugia for later colonization of surface biocrusts once mosses become established.

By investigating the influence of both hypolithic niche and the presence of moss, our study highlights the possible overlapping roles of mosses and hypoliths in shaping biocrust bacterial community composition. The results presented here corroborate the influence of dominant photoautotrophs, like moss, on community composition (Maier et al., 2018). Overall, community composition is heavily impacted by hypolithic niche: teasing apart communities associated with one biocrust type (moss or microbial) within niche (surface or hypolith) allowed us to differentiate the potential effects of these factors. This approach revealed that hypolithic spaces appear to harbor a unique bacterial community that is relatively insensitive to the presence of moss. At our study site, this hypolithic community is distinct from that in surface biocrusts that do not include moss; however, the effect of hypolithic niche is diminished in comparisons of moss biocrusts from hypolithic and surface habitats. If moss biocrusts and hypoliths are partially redundant in their bacterial community composition due to similarities in the physical microhabitats that they create, this may have implications for the design and implementation of projects aiming to restore biocrusts in the presence of historical or ongoing disturbance.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in NCBI SRA, NCBI Accession No. PRJNA577884.

AUTHOR CONTRIBUTIONS

KF, JJ, and PV contributed to the conceptual development and design of the study, and wrote sections of the manuscript. JJ collected samples, completed all lab work prior to sequencing, and wrote the first draft of the manuscript. KF performed assembly and analyses. All authors contributed to manuscript revision, read and approved the submitted version.

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Halophilic Algal Communities in Biological Soil Crusts Isolated From Potash Tailings Pile Areas

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Mining potash for fertilizer produces vast amounts of highly saline waste that is deposited in potash tailings piles. Rainfall washes the salts out, affecting the surrounding ecosystems. Only salt-tolerant organisms occur close to the piles, since other species cannot survive in these hypersaline conditions. Halophytic plant communities around tailings piles have been well investigated. However, studies exploring the biodiversity of the space behind the vegetation line that separates suitable salinities for plant growth from hostile conditions are rare. On top of the tailings piles, only micro-organisms thrive. This study, for the first time, explored the microalgae and cyanobacteria in biocrusts that inhabit potash tailings pile areas between the vegetation line and the pile body. Two biocrust types were studied: young biocrusts at or close to the tailings piles, and established biocrusts situated in the near surroundings. The estimated abundance of green algae, cyanobacteria and diatoms was studied using a direct microscopy approach, followed by the isolation and establishment of unialgal strains and morphological species determinations. Soil environmental characteristics were analyzed. Soil samples showed EC_{Sat} (electrical conductivity) values that drastically exceeded the scales commonly used to describe soil salinity, indicating extremely saline conditions. Indeed, the isolate composition was shaped by soil salinity parameters. Soil EC_{Sat} of young biocrusts tended to be higher than in established biocrusts, and filamentous green algae were most abundant. In contrast, established biocrusts tended to have a lower EC_{Sat} and were mostly dominated by filamentous cyanobacteria. Algal and cyanobacteria isolate composition differed significantly in young and established biocrusts, although the species number did not. Some of the salt-tolerant algal strains are assumed to be candidates for the formation of artificial biocrusts on the surface of the tailings. Attempts to "green" the piles by the establishment of higher plants to trap rainwater and therefore reduce the salt-output are difficult to apply. Plants require a thick layer of substrate to reduce the salinity, and the substrate easily erodes on the steep slopes. However, microalgae isolated from potash tailings pile areas seem promising, since they can survive on a thinner substrate layer and are already adapted to these hypersaline conditions.

Keywords: biocrusts, secondary salinization, salt tolerance, green algae, cyanobacteria, diatoms

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INTRODUCTION

Anthropogenic salinization is increasingly affecting ecosystems worldwide. The main drivers are human activities such as extensive agriculture, inadequate water management, mining and the use of de-icing salts. A high ion load, mostly Na^+, Ca^{2+}, Mg^{2+}, Cl^-, $CO_3{}^{2-}$,$HCO_3{}^-$, and $SO_4{}^{2-}$ (Williams, 1987) alters the flora and fauna of wetlands (Herbert et al., 2015) and freshwater systems (Cañedo-Argüelles et al., 2013; Kefford et al., 2016; Kaushal et al., 2018), and further affects the groundwater (Kloppmann et al., 2013) as well as terrestrial habitats such as grasslands (Pan et al., 2015) and farmland (Wang and Li, 2013). In addition to ecological problems, land and water salinization has socio-economic effects (Gorostiza and Saurí, 2019).

One of the main sources of salinization in Europe is potash mining (Cañedo-Argüelles et al., 2013). During the mining process, valuable components (mostly KCl and $MgSO_4$) of the potash ore are separated from unusable salt residues (mostly NaCl), generating liquid and solid salt residues. The brine is piped into streams and the solid overburden is deposited in tailings piles. Further, the pile waste water (up to 330 S_A, about 10 times that of marine sea water) leached by rainfall is either collected and discharged into streams or leaches out unhindered. Together, these processes lead to a high Na^+ and Cl^- output from potash tailings piles into the surrounding environment.

As a consequence, the load of salt ions in water bodies significantly affects the biotic communities, most often described as a decline in aquatic macroinvertebrate and fish diversity (Braukmann and Böhme, 2011; Arle and Wagner, 2013; Ladrera et al., 2017). Terrestrial habitats are also affected by potash tailings piles, and many studies have described the diversity of halophilic fauna and especially flora that replaced native species. Halophytes such as *Aster tripolium, Salicornia europaea*, and *Suaeda maritima* (Garve and Garve, 2000; Siefert et al., 2006; Woch and Trzcińska-Tacik, 2015), mainly occur in salt marshes along temperate coastal regions and only rarely inland, are often found next to potash tailings piles, underlining the saline character of these sites. Several habitats that emerged in conjunction with potash mining have even been designated as conservation areas because of the halophilic organisms.

In central Germany, dozens of potash tailings piles, some of them more than 200 m high, form the landscape next to various pile residues from previous smaller-scale mining. These large potash tailings piles are visible from a distance because of their unique appearance and lack of vegetation. Even though halophytes tolerate considerable concentrations of salt, the salinity of bare spoil material is too high to allow halophytes to colonize the piles. Often, a sharp vegetation line can be observed around the piles, following the prevailing salinity gradient. At higher salinities, only micro-organisms are able to thrive, but only a few studies have addressed their occurrence (Eilmus et al., 2007; Korsakova et al., 2013; Olsson et al., 2017). Therefore, the present study provides the first description of terrestrial micro-algal communities on potash tailings pile sites, which have so far been neglected.

Terrestrial algae are ecological key organisms in so-called biological soil crusts (biocrusts). These biocrusts are formed by a consortium of living organisms, which besides algae and cyanobacteria consists of heterotrophic bacteria, fungi, lichens and bryophytes, and their byproducts, creating a topsoil layer of inorganic particles bound together by organic materials. Biocrusts can be characterized as "ecosystem engineers" that form water-stable, aggregated surface layers. These layers have important multi-functional ecological roles in primary production, mineralization, bioweathering, dust trapping, and the stabilization of soils, slopes and entire landscapes, thereby affecting the nutrient and hydrological cycles across several scales (Weber et al., 2016). Consequently, biocrusts have their greatest impact in nutrient- or water-poor habitats that are little influenced by higher plants, often dominating regions with harsh, mostly arid environmental conditions worldwide (Bowker et al., 2010; Weber et al., 2016; Felde et al., 2017).

Biocrusts have been described on mining residues such as abandoned sand-mine areas (Doudle et al., 2011; Williams et al., 2019) and gold-mine tailings (Nyenda et al., 2019), as well as zinc- and lead-mine spoils (Trzcińska and Pawlik-Skowrońska, 2008). Thus, biocrusts can even cope with artificial and toxic conditions. Potash tailings piles, however, have not yet been studied, but various terrestrial algae inhabit other saline ecosystems (Tsujimura et al., 1998; Vinogradova and Darienko, 2008), although comprehensive studies are still rare.

Microalgae and cyanobacteria in biocrusts are well adapted to their often extreme habitats. A key factor for terrestrial phototrophic micro-organisms is water availability, which is strongly influenced by salinity. Although desiccation effects are physiologically closely connected to those of salinity, both stressors reflect two different forms of water deprivation. While under salt stress conditions, algal cells are often still in contact to liquid water of diminished water potential, desiccation leads to strong cellular dehydration. However, both drought and salt stress protective mechanisms work similarly. Desiccation tolerance is given by the formation of water-holding structures such as thick extracellular mucilage layers by the excretion of exopolymeric substances (EPS) and the formation of biofilms, cell colonies and aggregates, Karsten and Holzinger (2012). In turn, EPS have been suggested to have a salt-buffering effect (Chen et al., 2006; Lan et al., 2010; Rossi et al., 2018). Gustavs et al. (2010) showed that some terrestrial algae are able to produce organic osmolytes, which play another important role in desiccation tolerance and in turn is a common mechanism of halotolerant algae to tolerate high salinities (Karsten, 2012). Consequently, terrestrial algae in biocrust communities could have the potential to thrive in the highly saline potash tailings pile sites.

These salt-tolerant microalgae could further be candidates for restoration of the potash tailings piles by artificial biocrust formation. Recent studies reported the successful application of artificial biocrust formation through the inoculation of cyanobacteria in different habitats (e.g., sand dunes) to promote revegetation and restoration of degraded areas (Wang et al., 2009; Doudle et al., 2011; Lan et al., 2014). In the present case, a biocrust cover could potentially trap rainwater and thus reduce the harmful and uncontrolled salt erosion. However, selection of suitable algal isolates for artificial crust formation is crucial for the success of restoration (Román et al.,

2018; Giraldo-Silva et al., 2019). Consequently, microalgae and cyanobacteria that are already adapted to the tailings pile conditions might be the most suitable taxa.

Therefore, this study aimed, for the first time, to identify, describe and isolate halotolerant biocrust microalgae and cyanobacteria in the environment nearest to potash tailings piles. Two methodological approaches were used to characterize the algal composition, direct microscopic observation of biocrust material, and a culture-dependent approach including purification and establishment of clonal isolates. The study focused on two different biocrust types that were observed during fieldwork, and that represent the soil salt gradient from the vegetation line to the tailings pile body: first, established biocrusts closer to the vegetation line, and second, young biocrusts found on top or close to the potash tailings. Based on these field observations, we hypothesized that a smaller number of species should occur in young biocrusts, due to the higher salt stress along with a larger number of salt-tolerant taxa compared to established crusts.

MATERIALS AND METHODS

Study Sites and Terrestrial Algal Communities

The sampling was conducted at five different potash tailings pile sites in Lower Saxony and Saxony-Anhalt, Germany (**Table 1** and **Figures 1**, **2**). In both regions the mean annual temperature (1981–2010) is 9.3°C (Dwd, 2018). Saxony-Anhalt is generally drier with a mean annual precipitation of 579 mm (Mule, 2017) than Lower Saxony (787 mm). Three of the selected potash tailings piles derived from potash pits closed in the 1930s. These tailings piles were unprotected for approximately a century, which allowed uncontrolled leaching and a reduction in size (today up to 2 m in height) since their formation.

The site in Shreyahn (SY) features three isolated potash tailings pile residues, with a pond in the former pit, and is situated between a small forested area and farmland. It is part of a protected area and the largest "salt lick" of Lower Saxony. Facultative (e.g., *Aster tripolium*, *Glaux maritima*, *Puccinellia distans, Trifolium fragiferum*) and obligate halophytes (*Spergularia marina*) are present in the surrounding area (Jeckel, 1977). Well-developed biocrust communities including lichens and mosses cover primarily areas closest to of the potash tailings piles and are occasionally interspersed with small halophytes. Young green-algal biocrusts can form smaller patches directly on potash tailings pile material at the pile bottoms.

As in Shreyahn, the sampling site in Klein Oedesse (OD) is a part of a protected area (NSG Schwarzwasserniederung). Located in a woodland tract, the potash tailings pile is adjacent to a shallow pond, with halophytes such as *Atriplex longipes*, *Glaux maritima*, *Plantago maritima*, *Sueda maritim*a, *Trifolium fragiferum* and others in the surroundings (Garve and Garve, 2000). Sparse, grasses and a juvenile pine tree grow directly on the tailings pile. Well-developed biocrusts could be found around the bottom of the tailings pile. Since the tailings pile is easily accessible, trampling regularly interrupted the biocrust cover. At one spot, a greenish algal cover was observed directly on top of the tailings pile.

In Wietze (WT), an elongated area in the midst of a residential district includes both bare (in the front) and naturally revegetated potash tailings pile residues, indicating a successional gradient. Due to the absence of protected species, this site is not covered by conservation regulations. However, the halophytes *Gypsophila scoronerifolia* and *Hymenolobus procumbens* have been reported (Garve and Garve, 2000). Bare spots among the grass-dominated vegetation were occupied by well-developed biocrusts, and a thin and greenish algal crusts was found at the margin of the vegetation line facing the pile body.

In contrast to the sites described above, two of the tailings piles studied are considerably higher, cover larger areas and are generally younger in age (see **Table 1**). In Teutschenthal (TT) there are two large potash tailings piles, one of them studied here, shaping the district "Teutschenthal-Bahnhof" and creating a large saline area with both facultative (*Aster tripolium, Cochlearia danica*) and obligate (*Salicornia europaea*, *Suaeda maritima*) halophytes (Garve and Garve, 2000). Established biocrusts could be found in front of the vegetation line, some meters distant from the pile body. In addition, a young algal biocrust was situated on flat material at the pile bottom, next to a water catchment basin. At a second large tailings pile site (NN) both young and established biocrusts were found.

Sampling

Biocrust samples were collected with plastic Petri dishes (5.5 cm ø) using a spatula and closed with Parafilm®. In addition, a soil

TABLE 1 | Description of the potash tailings pile sites.

Name	Site description	Coordinates	Max. height (approx.)	Area (approx.)	Potash extraction period
Oedesse (OD)	One pile	52.384009°N 10.220794°E	2.0 m	15 × 20 m	1913–1925
Shreyahn (SY)	Three piles, approx. 10 m in distance1916–1926	52.931452°N 11.076959°E	1.5 m	80 × 30 m	1916–1926
Wietze (WT)	Several residues, with a N-S gradient (old-young)	52.643704°N 9.863592°E	0.8 m	150 × 30 m	1908–1923
Teutschenthal (TT)	Two piles, one of them studied herein	51.467324°N 11.770714°E	60 m	650 × 400 m	1906–1982
NN	Not further described				

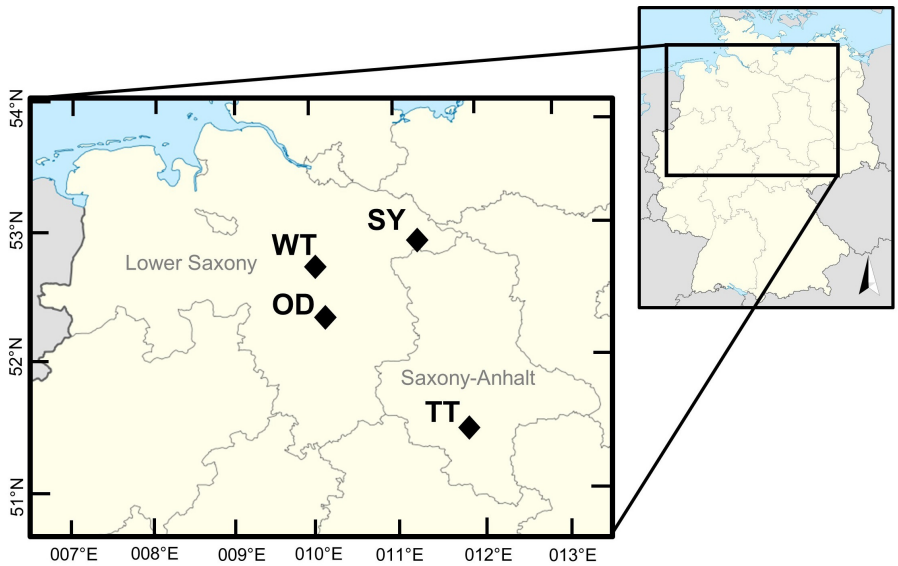

FIGURE 1 | Sampling locations in Lower Saxony and Saxony-Anhalt (OD, Oedesse; SY, Shreyahn; TT, Teutschenthal; WT, Wietze; NN not included).

sample of approximately 5 cm× 5 cm× 3 cm from the upper layer of a bare spot close to the respective biocrust was collected and stored in LDPE plastic bags. If no bare spot was available, the biocrust with its adhering soil (3 mm) underneath was removed and the soil below was sampled as described. After sampling, the Petri dishes and plastic bags were opened to air-dry the samples and were then stored closed in a dry, dark place.

Soil Characterization

To determine the electrical conductivity (EC), 10 g air-dried soil (<2 mm) was mixed with 50 ml aqua deion (<5 $\mu S\ cm^{-1}$). After shaking for 1 h followed by 30 min of sedimentation, the EC of the supernatant was measured with an EC meter (Seven Multi^{TM}, Mettler Toledo, Schwarzenbach/Germany, in Lab 731 probe). The measurement values were used to calculate the saturation extract EC, with the following equation:

$$EC_{Sat} \approx EC^{*}6.4\ [mS\ cm^{-1}\ ]\ \text{(Rowell, 1997)}.$$

For pH_{CaCl2} measurement, 2 g air-dried soil (<2 mm) and 8 ml 0.01 M $CaCl_2$ were mixed, shaken for 5 min, and incubated for 2 h. The pH_{CaCl2} was measured with a pH meter (Seven Multi, Mettler Toledo, Schwarzenbach/Germany, inLab Expert Pro probe).

To determine tOC, tN and tS, first, inorganic carbon was removed with 10% HCl in the carbonate-containing samples. Then, four replicates of 300 mg of ground soil were each packed tightly into tin boats and analyzed with a Vario El III (Elementar, Langensbold/Germany). The C:N ratio was calculated referring to the molecular mass.

Ion chromatography was conducted to determine cations (Na^+, K^+, Mg^+, Ca^{2+}) and anions (Cl^-, $SO_4{}^{2-}$) in aqueous soil extracts. First, 3 g air-dried and ground soil was mixed with 30 ml aqua deion. After 15–18 h of incubation at 70°C in a water bath, samples were filtered through a Whatman^{TM} GF-6 glass-fiber filter (Whatman/GE Healthcare) and the sample vessel and filtration apparatus were washed with hot aqua deion and added to the sample. After cooling to room temperature, the filtrate was filled with aqua deion to a volume of 50 ml. For anions, samples were analyzed by ion chromatography (Metrohm AG, Switzerland) with chemical suppression, inline dialysis and inline dilution using a Metrohm Metrosep A Supp 5 separation column (eluents: 3.2 mM $NaCO_3$, 1.0 mM $NaHCO_3$; 0.7 ml min^{-1} flow rate; guard: Metrosep A Supp 4/5). Samples for cation determinations were diluted and acidified with 20 µl 1 M HNO_3 before analysis by ion chromatography (Metrohm AG, Switzerland) with inline filtration using a Metrohm Metrospec C 4 – xx/4.0 column (eluents: 1.7 mM HNO_3, 0.7 mM $C_7H_5NO_4$; 0.9 ml min^{-1} flow rate, guard: Metrosep C4 Guard/4.0). Each test series was calibrated, using internal standards.

Algae Culturing and Identification

Dominant algal groups were identified by means of direct light microscopy. Small pieces collected from the surface of dried biocrust material were mixed with a few drops of tap water, rewetted for 30 min, and observed under an Olympus BX51 light microscope. In order to classify the algal groups, the estimated abundance of diatoms, green algae and cyanobacteria were classified using the following scale: 0 = absent, 1 = observed once, 2 = rare, 3 = occasionally, 4 = frequent, and 5 = abundant (Schulz et al., 2016). For green algae and cyanobacteria, both the filamentous and non-filamentous stages were listed separately as a percentage of identified algae, e.g., 20% unicellular and 80% filamentous green algae. Diatom morphotypes were also identified by direct light microscopy.

For the preparation of enrichment cultures, small pieces of dried biocrusts were placed in a petri dish filled with MBBM + V

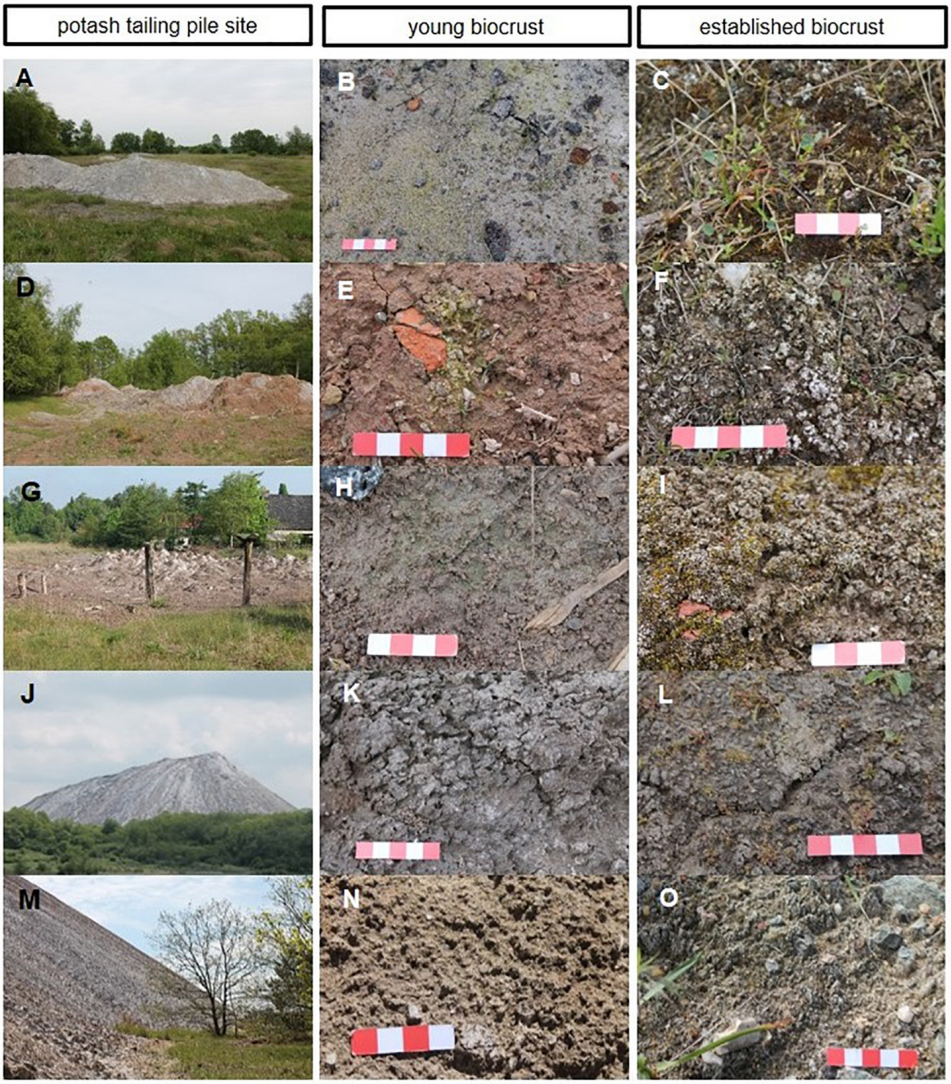

FIGURE 2 | Potash tailings piles sites and the respective young and established terrestrial algal communities. **(A–C)** Shreyahn; **(D–F)** Oedesse; **(G–I)** Wietze; **(J–L)** Teutschenthal; **(M–O)** NN; one red or white square of the scale bar indicates 1 cm.

(Bischoff and Bold, 1963; modified by Starr and Zeiskus, 1993) modified by the addition of 3% NaCl and solidified using 1.5% Difco© agar. After approximately 4 weeks, single colonies were transferred to fresh solid medium. Green algae were placed on solid 3N-BBM + V (Bischoff and Bold, 1963; modified by Starr and Zeiskus, 1993) +3% NaCl, whereas Cyanobacteria were transferred to solid BG-11 (Rippka and Herdmann, 1992) +3% NaCl. This step was repeated until unialgal, clonal isolates were established. Diatoms did not grow in this culture sequence. All cultures were kept at 15–20°C in a 16:8 light:dark cycle and 30 μmol photons m^{-2} s^{-1}.

For morphological species determination, isolates were observed under an Olympus BX51 light microscope, mainly using the identification key of Ettl and Gärtner (2014). Photomicrographs were taken with the cellSense Entry imaging software (Olympus). The taxon designation was compared with the AlgaeBase database (Guiry and Guiry, 2020) and renamed if necessary, in order to report the latest, taxonomically accepted species and genus names.

A list of taxa was prepared, using the culture-dependent results. In the **Supplementary Table S1** it was combined with the culture-independent results but all further and statistical analyses were based on the taxa isolated. The sum of the present taxa of each site reflects the number of taxa per site.

Statistical Analyses

The following calculations were performed with the program R (Version 3.3.2). Analyses of Similarity (ANOSIM) were performed to calculate a correlation between species composition and single categories, i.e., sampling site, biocrust type (young biocrust, established biocrust) and tailings pile age class (recent, older). First, co-correlations between the environmental factors

as well as correlations between the main algal groups and environmental factors were calculated by Pearson parametric correlation tests. One-Way Permutational Multivariate Analyses of variance (perMANOVA, Jaccard distance) were performed to test for environmental parameters that significantly affected the composition of isolates. Correlations of single environmental factors with the two biocrust types were analyzed using a Mann-Whitney *U* test. A non-metric multidimensional scaling of a dissimilarity matrix (Jaccard distance) of the algae presence/absence list was performed using the isoMDS function of the MASS (Venables and Ripley, 2002) package. If not stated otherwise, all calculations were performed using the vegan package (Oksanen et al., 2017) or defaults of R.

RESULTS

Soil Characteristics

In total, EC_{Sat} ranged between 49–954 mS cm^{-1} (**Table 2**). The median and maximum EC_{Sat} of young biocrusts (159 mS cm^{-1}, 954 mS cm^{-1}) were higher than those of established biocrusts (105 mS cm^{-1}, 278 mS cm^{-1}), although this trend was not statistically significant ($p = 0.056$). The dominant anion was SO_4^{2-}. Ca^{2+} was the dominant cation in most samples, although in two soil samples Na^+ was present in a higher proportion than Ca^{2+}. The EC_{Sat} was positively correlated with the concentrations of Cl^-, SO_4^{2-}, Na^+, K^+, Mg^{2+} in the soil. The soil pH_{CaCl2} ranged from neutral to moderately alkaline (7 and 9). There was no significance difference in soil pH_{CaCl2} between young and established biocrusts. Further, soil pH_{CaCl2} was positively correlated with Na^+, SO_4^{2-} and tOC (Pearson correlation, results not shown). Total organic carbon and total nitrogen contents and the ratio between them varied widely among the sites, but no differences between young and established biocrusts could be observed.

EC_{Sat} tended to differ, and the concentrations of Na^+ differed significantly between the soil of young and established biocrusts (**Table 3**).

Estimation of Major Algal Group Composition and Abundance

The composition and abundance of major algal groups in young and established biocrusts was estimated by direct microscopy (**Figure 3**, details in **Figures 4**, **5**). Diatoms (**Figure 4**) were generally rare to absent. Most of the established biocrust samples were dominated by cyanobacteria; green algae were more abundant than cyanobacteria in only four samples, and one sample showed an equal distribution of all three algal groups. Regarding the organization level, mostly unicellular green algae were observed, whereas filamentous green algae were present in five of 11 samples. Most cyanobacteria in established biocrusts were filamentous, and unicellular cyanobacteria were observed in only three samples. In contrast, all samples from young biocrusts were dominated by green algae, and two of these samples contained a larger share of filamentous organisms than of non-filamentous. The maximum cyanobacteria frequency was "occasional," two samples lacked cyanobacteria. In four samples of young biocrusts, diatoms occurred; in one sample they were rare, and in the other samples, diatoms were observed only once.

Identification of Diatoms

Direct observations of dry biocrust material revealed in total six different morphotypes of diatoms: *Navicula* sp., *Hantzschia* sp., *Surirella* sp., *Luticola* sp. and *Luticola* cf. *nivalis* (**Figure 4**). Only *Surirella* sp. occurred in the cultures.

Identification of Green Algae and Cyanobacteria

Number of Taxa

In total, 73 algal and cyanobacterial taxa were identified by enrichment culturing and subsequent morphological identification. Up to 17 taxa were identified per site (**Figure 6**). Each established biocrusts sample contained on average 11 taxa and each young biocrust sample 10. There was no significant difference in species number between both biocrust types. In total, the two biocrust types had 25 taxa in common, whereas 32 taxa were only found in established biocrusts and 16 in young biocrusts, respectively.

Overall, the majority of isolated species were green algae whereas cyanobacteria were clearly less diverse. In five samples, no cyanobacteria were isolated. In regard to green algae, most algae belonged to the class Trebouxiophyceae, followed by Chlorophyceae. Interestingly, Ulvophyceae were most frequent in young biocrusts and were rarely found in established biocrusts. For Xanthophyceae, one taxon was recorded.

Algal and Cyanobacterial Isolate Composition

The isolate composition of green algae and cyanobacteria was determined by the culture-dependent approach (**Figure 7**). The most frequent green alga taxa during culturing were *Stichococcus bacillaris*, *Nannochloris* sp., *Bracteacoccus minor*, and *Diplosphaera chodatii*. Besides *S. bacillaris*, other species of this genus, *S. allas* and *S. exiguus,* were each found in several samples. Other frequent taxa were *Planophila terrestris*, *Spongiochloris excentrica*, *Myrmecia bisecta* and *Tetracystis* sp. Two filamentous green algae genera were observed, *Ulothrix* and *Pseudoclonium,* both of members of *Ulvophyceae*. *Leptolyngbya* sp., and *Nostoc* sp. were frequent filamentous cyanobacteria isolates.

Nostoc sp. was only present in established biocrusts and absent in young biocrusts. Likewise, *Parietochloris cohaerens* and *Diplosphaera chodatii* were found in all established biocrust sample sites whereas the latter only occurred once in young biocrusts. *Chloroidium ellipsoideum* and *Stichococcus* sp. 1 were also characteristic of established biocrusts. *Chlorella vulgaris* was more often found in young than in established biocrust samples.

Some of the morphologically identified taxa could only be observed by direct microscopy (**Supplementary Table S1**). This includes members of the genera *Mesotaenium* and *Gloeocapsa*. In addition, *Microcoleus vaginatus* was often observed by direct microscopy (**Figure 5**) but was mainly absent in the subsequent

TABLE 2 | Characterization of soil samples from young and established biocrusts in potash tailings pile areas (OD: Oedesse; SY: Shreyahn; TT: Teutschenthal; WT: Wietze; NN: not further described).

Crust type	Plot	EC_{Sat}	Na^+ [mg g^{-1}]	K^+ [mg g^{-1}]	Ca^{2+} [mg g^{-1}]	Mg^{2+} [mg g^{-1}]	Cl^- [mg g^{-1}]	$SO4^{2-}$ [mg g^{-1}]	pH	tON [mg g^{-1}]	tOC [mg g^{-1}]	C:N
Young biocrust	OD-2	193.92	4.96	0.05	7.57	0.22	4.48	22.27	8.092	12.49	172.30	11.83
	SY-2	114.88	2.32	0.06	8.67	0.36	0.00	26.88	8.306	21.85	543.62	21.33
	SY-3	807.04	19.18	0.12	6.43	0.96	3.04	55.08	8.938	26.31	463.90	15.12
	TT-4	954.24	11.90	1.19	10.19	3.70	23.02	34.11	8.281	13.14	133.77	8.73
	WT-4	142.72	1.45	0.04	8.38	0.13	0.00	23.50	8.408	9.89	391.26	33.93
	NN-3	158.72	2.70	0.13	0.11	0.02	3.34	0.69	7.029	13.23	80.57	5.22
	NN-5	145.28	1.19	0.03	8.91	0.04	1.46	21.18	7.149	9.26	94.55	8.76
	median	**158.72**	**2.70**	**0.06**	**8.38**	**0.22**	**3.04**	**23.50**	**8.281**	**13.14**	**172.30**	**11.83**
	range	**839.36**	**17.99**	**1.17**	**10.09**	**3.68**	**23.02**	**54.40**	**1.909**	**17.05**	**463.06**	**28.71**
Established biocrust	OD-1	105.28	4.50	0.05	8.34	0.33	0.02	30.55	8.264	13.94	584.30	35.92
	SY-1	48.64	0.11	0.02	8.06	0.34	0.00	20.21	7.969	25.84	424.08	14.07
	TT-1	202.24	2.36	0.09	8.27	0.22	1.12	23.60	8.488	44.40	172.22	3.32
	TT-2	129.28	1.11	0.05	8.53	0.26	0.00	23.81	7.76	24.96	272.40	9.35
	TT-3	273.28	3.88	0.06	8.59	0.24	2.57	24.95	8.81	13.16	118.92	7.75
	WT-1	122.56	0.11	0.02	9.29	0.05	0.00	22.25	8.124	10.55	158.74	12.89
	WT-2	95.81	0.31	0.05	26.64	0.40	0.00	21.78	7.92	9.21	272.82	25.38
	WT-3	82.69	0.33	0.06	8.91	0.27	0.00	21.68	8.134	14.44	264.52	15.70
	NN-1	89.34	0.18	0.05	8.55	0.04	0.00	20.38	7.053	34.24	86.79	2.17
	NN-2	79.81	1.87	0.45	n. d.	n. d.	2.28	0.45	7.116	27.65	244.89	7.59
	NN-4	278.40	2.52	0.05	9.97	0.02	3.39	23.85	7.239	11.09	89.42	6.91
	median	**95.81**	**0.33**	**0.05**	**9.10**	**0.14**	**0.00**	**21.78**	**7.92**	**13.16**	**158.74**	**7.75**
	range	**198.59**	**3.77**	**0.44**	**18.09**	**0.39**	**3.39**	**24.50**	**1.757**	**25.03**	**186.03**	**23.21**

Median and range in bold, tON, tOC, and C:N represent mean values (n = 4), n. d. = no data.

TABLE 3 | Results of screening for significant differences between young and established biocrusts regarding number of algae taxa (culture-dependent approach) and environmental parameters, by Man-Whitney *U* Test.

Parameter	W	*p*-value	Level of significance
Number of taxa	50	0.31760	
EC_{Sat}	17	*0.05556*	·
Cl^-	21	0.10720	
SO_4^{2-}	29	0.42520	
Na^+	16	**0.04412**	*
K^+	29	0.42520	
Ca^{2+}	46	0.31480	
Mg^{2+}	33	0.88680	
tON	51	0.28540	
tOC	38	1.00000	
pH	31	0.53600	

*Level of significance: *p < 0.05 (value bold), ·p < 0.1 (value italic).*

TABLE 4 | (A) Analyses of similarity to reveal significant differences of the present algal isolate composition between the sites, biocrust type (young, established) and potash tailings pile age classes. **(B)** Influence of abiotic conditions on algal isolate composition tested by PerManova; given are: F-value, R2-value (which corresponds to the variation in the isolate composition explained by the respective factor) and level of significance: *p < 0.05 (values in bold), ·p < 0.1 (values italic); df: degrees of freedom.

	R	*p*	Level of significance
(A) ANOSIM			
Site	0.07965	0.237	
Crust type	0.2336	**0.021**	*
heap age class	0.04561	0.227	

	df	*F*	R2	*p*-value	Level of significance
(B) One-way perMANOVA					
EC_{Sat}	1	1.3351	0.07702	*0.072*	·
Cl^-	1	1.3418	0.07738	**0.025**	*
SO_4^2	1	1.3288	0.07668	*0.073*	·
Na^+	1	1.4224	0.08164	**0.023**	*
K^+	1	1.0863	0.06358	0.283	
Ca^{2+}	1	1.3058	0.08008	*0.051*	·
Mg^{2+}	1	1.0521	0.06554	0.360	
C/N	1	0.9617	0.0567	0.554	
tON	1	1.0617	0.06223	0.357	
tOC	1	1.0967	0.06415	0.303	
pH	1	1.4618	0.08371	**0.041**	*

enrichment culture; in some samples this was the case for *Nostoc* sp. as well.

Dissimilarities in the presence of algal isolates between samples are shown in the nMDS plot (**Figure 8**). First, no clustering of samples from similar sites could be observed, indicating no site-specific isolate compositions; accordingly, this fact was proved to be non-significant (**Table 4**). Grouping the sites by tailings pile age (recent, older) also indicated no significant differences. However, grouping the samples by biocrust type revealed a distinct division, in the lower left of the graph for young and the upper right for established biocrusts. Analysis of similarity confirmed a significant difference in the isolate composition between the young and established biocrusts (**Table 4**).

Algal and Cyanobacterial Composition and Soil Characteristics

The major algal groups, cyanobacteria, green algae and diatoms that were studied with direct microscopy, showed no correlation between their estimated abundance and the EC_{Sat} of the soil. However, significantly more filamentous green algae were found with increasing EC_{Sat} of the soil. In contrast, filamentous cyanobacteria showed a negative trend. Further, EC_{Sat} and SO_4^{2-} concentrations of the soil tended to influence the total composition of algal isolates, whereas Na^+ and Cl^- concentrations and pH_{CaCl2} of the soil were found to significantly affect the isolate composition (**Table 4**). All other tested concentrations of ions, tOC and tON in the soil did not correlate with the present algal and cyanobacteria isolate composition.

DISCUSSION

Soil Characteristics Reflect Extremely Saline Environments

Soils with an EC_{Sat} of >16 mS cm^{-1} are defined as very highly saline soils (Whitney, 1988). According to this definition, the soils in the present study can be regarded as "extremely saline" soils (**Table 2**). Until now, only one study has recorded terrestrial algae in soils with an EC_{Sat} up to 260 mS cm^{-1} (Tsujimura et al., 1998). Other studies on biocrusts on saline soils reported only sites with EC_{Sat} values <30 mS cm^{-1} (e.g., Fox et al., 2009; Kidron, 2016; Sandoval Pérez et al., 2016; Schulz et al., 2016; Zhou et al., 2016; Ghiloufi et al., 2017; Kakeh et al., 2018). In the present study, however, much higher ranges of EC_{Sat} (up to 950 mS cm^{-1}) were documented, emphasizing the extreme soil salinities of the sampling sites.

Na^+ and Cl^- were not the prominent ions measured in the aqueous soil extracts of the present study. The main component of fresh potash mining residues, however, is NaCl. This distinct difference is explained by the lower sorption of these ions and consequently by their rapid leaching. The most dominant anion in the soil solution was SO_4^{2-}. Likewise, SO_4^{2-} was the main ion in a nickel-mining seepage with very high EC (Van Dam et al., 2014). A high sulfate content in soils typically indicates gypsum or anhydrite. Besides addressing the absolute amounts of ions, which also mirrors the EC, the ion ratios are crucial for physiological processes in cells. According to Albrecht (1975) the optimal cation balance for plant growth in agricultural soils is: Ca 60–75%, Mg 10–20%, K 2–5%, Na 0.5%, H 10% and other cations 5%. Thus, there was a strong deficiency of Mg and K in the tested soil samples, whereas Na had a considerably higher share. Taken together, these findings indicate the extreme, chemically unbalanced and rather artificial soil conditions due to potash mining, which thus comprise unique habitats for organisms.

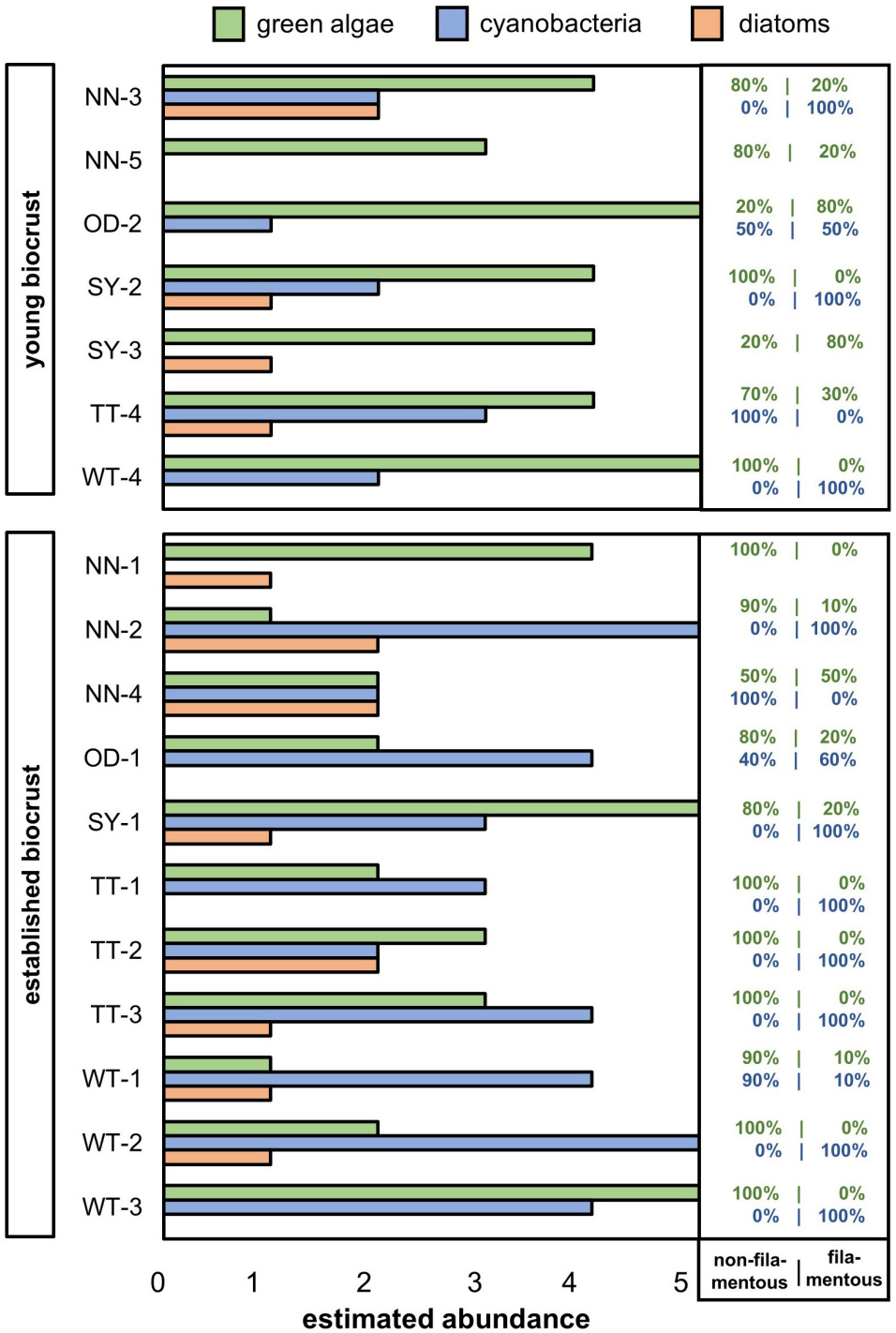

FIGURE 3 | Estimated abundance of major algal groups in young and established biocrusts from potash tailings pile sites (OD, Oedesse; SY, Shreyahn; TT, Teutschenthal; WT, Wietze; NN, not further described) determined by direct microscopy of biocrust material. The estimated percentage of filamentous and non-filamentous green algae (green) and cyanobacteria (blue) are noted on the right; all observed diatoms were non-filamentous.

Bare potash tailings pile material is considered to be very low in organic matter. Since this material is a residue from a mined mineral, it is not comparable to soils on the earth's surface that were formed by complex biological processes, leading to enrichment of organic matter over long periods. The soil samples of young biocrusts collected at or very close to the pile body did not differ from established biocrusts in tOC. This might be caused by the suppression of fungal growth in alkaline soils, which

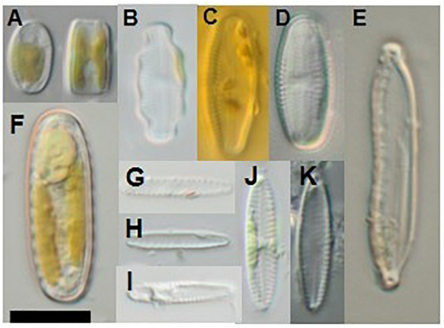

FIGURE 4 | Diatom morphotypes observed directly in rewetted biocrust material. **(A)** *Cymbellaceae*; **(B)** *Luticola* cf. *nivalis*; **(C,D)** *Luticola* sp.; **(E)** *Hantzschia* sp.; **(F)** *Surirella* sp.; **(G–K)** *Navicula* sp.; scale bar indicates 10 μm.

can cause a significant decrease of the soil microbial biomass (Weyman-Kaczmarkowa and Pędziwilk, 2000). However, in a broad study that analyzed biocrust samples of several countries in Europe, tOC was also found to vary strongly (Williams et al., 2018), which led the authors to the conclusion that tOC, among other soil parameters, is not suitable to describe crust developmental stages.

It is difficult to determine whether the biocrust emerged due to a previous input of organic matter from litter, or whether the organic carbon was accumulated predominantly from the photosynthetic activity of algae and cyanobacteria in the biocrust itself. Most likely a combination of both processes occurs, because biocrusts are known to strongly contribute to the C pools of soils (Sancho et al., 2017). Organic matter covering the tailings piles lowers the salinity of the surface and acts as a kind of buffer layer, which could be crucial for the establishment of a biocrust in such an extreme saline habitat.

Rich Algal and Cyanobacteria Isolate Composition

Methodological Considerations

Morphological identification based on the culture-dependent approach was especially challenging in this study, because some of the isolates may have an irregular morphology due to the saline culture medium, and most descriptions from the literature are based on isolates cultured in non-saline media. For this reason, several morphotypes could not be identified at species level. Due to the salt stressor, cells of one species could be shaped differently and be assigned to a different morphotype, thus leading to overestimation of the true biodiversity. In contrast, cells could lose special characteristics such as pyrenoid morphology due to the stressor, which might cause underestimation of the true biodiversity. This effect, termed phenotypic plasticity, has been described for both green algae and cyanobacteria subject to several stressors, such as temperature (Neustupa et al., 2008; Soares et al., 2013) as well as salinity (Darienko et al., 2015).

Organisms observed with direct microscopy could not be completely rewetted, which impeded species determination. Some taxa may have been present only as resting stages, which are often impossible to identify. For secure species identification, it is proposed to use polyphasic approaches including morphological, genetic and ecophysiological observations (Darienko et al., 2010), preferably in combination with methods that are not culture-dependent. Identification of diatoms was also challenging. In view of these difficulties, morphotypes were defined only to the highest taxon level possible using the methods in this study. For more precise species determination, diatoms must be observed on permanent slides or further details evaluated with scanning electron microscopy. However, diatoms were not a focus of this study, as they were comparatively rare at the study sites and are generally not regarded as crust-initiating organisms.

The morphological species determination provides a first insight of algae and cyanobacteria communities of biocrusts in potash tailings pile areas. For a more precise description of the taxon composition, a polyphasic approach is recommended.

Number of Morphologically Identified Taxa

In total, 73 microalgal and cyanobacterial taxa were observed with the culture-dependent approach, and six diatom morphotypes were identified by direct microscopy. This number is similar to the results of another study of microalgae and cyanobacteria in salt-affected soils that reported 74 species (Vinogradova and Darienko, 2008). In temperate regions, a lower taxa richness was observed for biocrusts in forests (Glaser et al., 2018) and sand dunes (Schulz et al., 2016). On the one hand, it was rather surprising to find more species in this extreme habitat than in forests or dunes, where the edaphic parameters are less challenging for algae. On the other hand, in the potash-tailings pile areas biocrusts face lower competitive pressure by higher plants, which could explain the relatively high number of taxa. Concerning biocrust types, there was no difference in species number between young and established biocrusts, which agrees with a study along a transect of a glacier forefield that also reported no differences between young and established biocrusts (Borchhardt et al., 2019). In contrast, a study in a non-saline region showed an increase in species number concerning green algae and cyanobacteria in biocrusts during their succession (Zhang et al., 2018). In summary, our hypothesis that the species number in young biocrusts is lower than in established biocrusts was not confirmed. This result underlines that even biocrusts of early successional stages can represent diverse algal communities, which is especially remarkable for the extreme environment of potash tailings piles.

Major Algal Group Estimated Composition Differed Between Biocrust Types

Filamentous microalgae can be regarded as crust-initiating, as they interweave soil particles and thus stabilize the surface. This form of organization seemed to be important for biocrusts on these tailings piles, as we found filamentous taxa in all samples. In young biocrusts this function was mainly accomplished by green algae, whereas in established biocrusts cyanobacteria were the most abundant filamentous organisms. In arid regions, cyanobacteria are generally regarded as the first organisms that occur during biocrust succession, followed by green algae

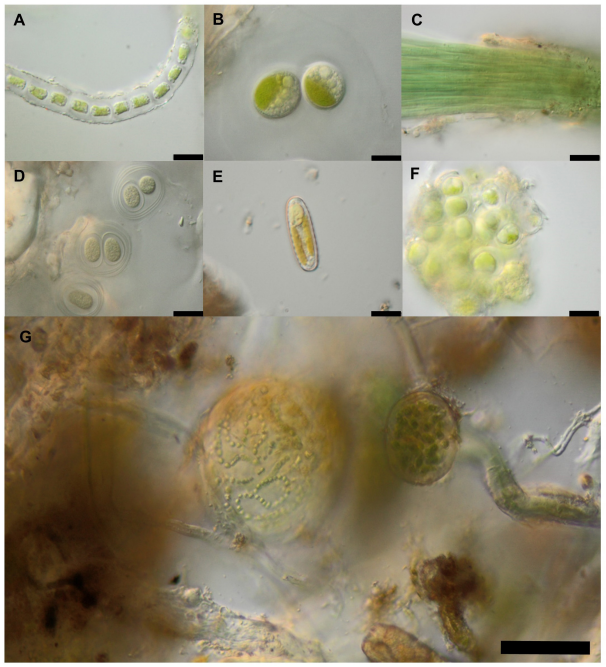

FIGURE 5 | Microphotographs of direct microscopy observations of biocrust material. **(A)** filament of *Ulothrix*-like algae; **(B)** *Mesotaenium* cf. *chlamydospora*; **(C)** *Microcoleus vaginatus*; **(D)** *Gloeocapsa* sp.; **(E)** *Surirella* sp.; **(F)** *Apatococcus lobatus*; **(G)** *Nostoc* sp. colonies and *Microcoleus vaginatus* filaments between soil particles (brownish). Scale bars indicates 10 μm **(A–F)** and 50 μm **(G)**.

(Belnap, 2006). This succession pattern seems to be more variable in temperate regions, since both green algae and cyanobacteria dominated biocrusts appear in habitats strongly disturbed by humans (Szyja et al., 2018). In our study, cyanobacteria were notably less abundant in young biocrusts compared to filamentous green algae, which might be explained by the specific environmental conditions. Interestingly, another study in temperate regions on mine dumps, from lignite mining, also found a low abundance of cyanobacteria in initial biocrusts, in this case attributed to very low pH values (Lukešová, 2001). Cyanobacteria are normally missing in habitats with an acid pH range (Brock, 1973). However, all soil samples of the present study were within the pH_{CaCl2} range preferred by cyanobacteria. There was no difference between pH_{CaCl2} and biocrust types, whereas the estimated abundance of cyanobacteria was much higher in established biocrusts. Thus, the pH_{CaCl2} cannot explain the lack of cyanobacteria in young biocrusts. The estimated abundance of filamentous green algae showed a positive correlation with EC_{Sat}. In contrast, the estimated abundance of filamentous cyanobacteria identified by direct microscopy tended to indicate a negative correlation with EC_{Sat}. Tsujimura et al. (1998) found a similar trend for cyanobacteria but only for *Nostocales*, whereas *Oscillatoriales* showed a negative trend. However, this subdivision was not evaluated with respect to estimated abundance in the present study.

Algal Isolate Composition Is Shaped by Soil Salinity and pH

The most crucial environmental factor in potash tailings pile areas is salinity. The concentrations of Na^+ and Cl^- in the soil solution had a significant effect on algal isolate composition, which can be explained by the pronounced toxicity of both ions to all living cells (Kirst, 1990 and literature cited therein). In contrast, Ca^{2+} is not toxic and is important as a messenger in salt-tolerance processes (Kirst, 1990 and literature cited therein), and therefore this cation could be important for the composition of green algal and cyanobacterial isolates. K^+ is compatible with the cell metabolism and did not affect the composition of green-algal and cyanobacterial isolates. However, the ratio

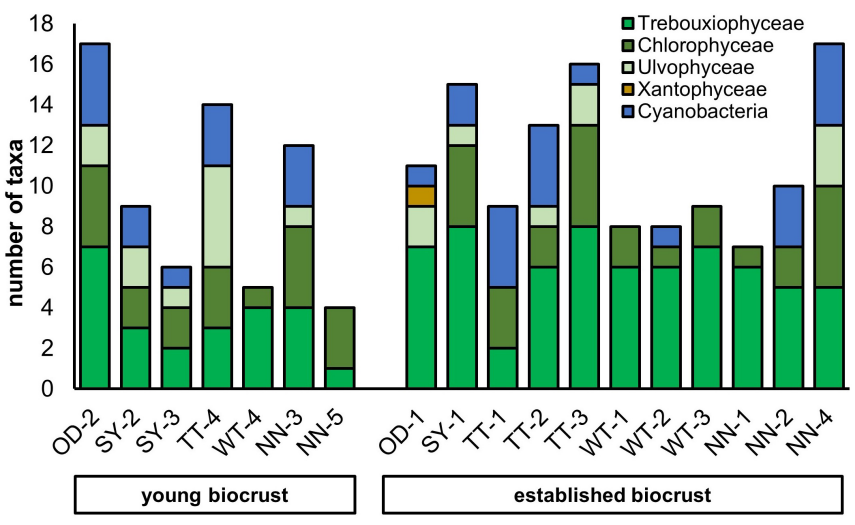

FIGURE 6 | Number of taxa isolated from biocrust samples (OD, Oedesse; SY, Shreyahn; TT, Teutschenthal; WT, Wietze; NN, not further described) grouped by biocrust type divided in algae classes and cyanobacteria.

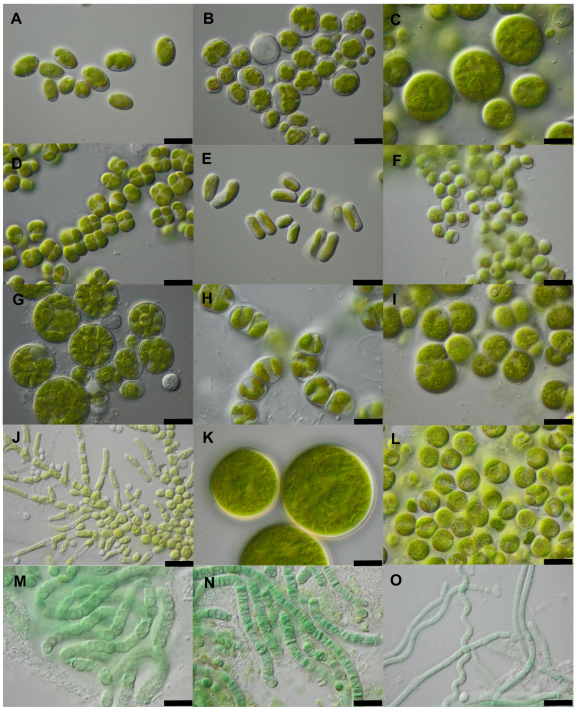

FIGURE 7 | Green algae and cyanobacteria isolated from biocrusts in potash tailings pile areas. **(A)** *Chloroidium ellipsoideum* (WT-1); **(B)** *Parietochloris cohaerens* (NN-4); **(C)** *Spongiochloris excentrica* (TT-5); **(D)** *Diplosphaera chodatii* (NN-5); **(E)** *Stichococcus allas* (NN-4); **(F)** *Chlorella* sp. 1 (SY-1); **(G)** *Borodinellopsis texensis*; **(H)** *Ulothrix aequalis* (TT-3); **(I)** *Planophila laetevirens* (OD-2); **(J)** *Pseudendoclonium* sp. (TT-5); **(K)** *Bracteacoccus minor* (OD-3); **(L)** *Myrmecia bisecta* (TT-3); **(M)** *Nostoc* sp. (SY-1); **(N)** *Pseudophormidium edaphicum* (NN-8); **(O)** *Leptolyngbya* sp. (NN-5). Scale bars indicate 10 μm **(A–N)**, and 20 μm **(O)**.

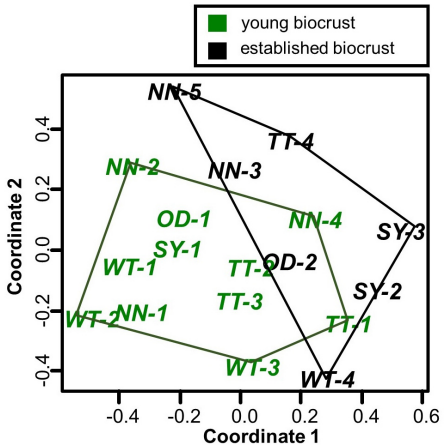

FIGURE 8 | nMDS plot showing the present morphotaxa composition of green algae and cyanobacteria isolated from young and established biocrusts sampled in potash tailings pile environments (OD, Oedesse; SY, Shreyahn; TT, Teutschenthal; WT, Wietze; NN, not further described) polygons show the biocrust types; stress = 0.179 and Nostoc sp. were prominent cyanobacteria taxa.

between the toxic Na^+ to the nutrient K^+ is important, since K^+ is likely exchanged with the toxic Na^+ in cells, as reported for cyanobacterial cells by Hagemann (2011). Even though SO_4^{2-} was the prominent ion, there was no significant effect on the composition of algal and cyanobacterial isolates, since it was not the growth-limiting nutrient. In a study on *Chlorella* sp. increased SO_4^{2-} concentrations showed lower toxicity than Cl^- (Van Dam et al., 2014). This further explains why Cl^- is more important for the composition of algal and cyanobacterial isolates. Generally, the lack of a significant effect of EC_{Sat} on algal isolate composition underlines the importance of individual ions. Salinity could also explain the differences in the composition of algal isolates between young and established biocrusts, since the concentration of Na^+ in the soil solution was significantly different and EC_{Sat} also tended to differ between biocrust types. In conclusion, species found in young biocrusts may tolerate higher soil salinities. Still, there might be differently halotolerant species among the microalgae. The three dimensions of a biocrust offer numerous microhabitats with distinct environmental conditions. Less salt-tolerant organisms could be situated in the middle layer, sheltered by salt-tolerant and polysaccharide excreting algae. It would be very interesting to evaluate the salt tolerance range of the taxa isolated here in future and to compare the response patterns of isolates from both young and established biocrusts.

Another factor that significantly affected the composition of algal isolates was soil pH_{CaCl2}. Different results regarding pH_{CaCl2} and algal community composition have been reported. Some studies showed a significant effect of pH (Castillo-Monroy et al., 2016) and others found no correlation (Schulz et al., 2016; Glaser et al., 2018). Soil pH is crucial for the availability of plant nutrients. Potassium and sulfur are easily bioavailable in slightly acidic to strongly alkaline soils, whereas other nutrients such as nitrogen, phosphorus and some trace elements show a lower bioavailability in alkaline soils. Especially phosphorus has decreasing bioavailability with increasing alkalinity of soils. Several soil samples in this study were alkaline, which leads to decreased bioavailability of several other nutritional elements (e.g., Ca^{2+}, Mg^{2+}, K^+, Fe^{2+}). This may explain why the taxa diversity depends on pH.

Present Taxa Mirror Extreme Soil Salinity

Most diatom morphotypes identified by direct microscopy are known terrestrial taxa. *Luticola nivalis* was previously found in biocrust samples (Kidron et al., 2012; Borchhardt et al., 2017), and several species of *Hantzschia* sp., *Luticola* sp. and *Navicula* sp. were members of biocrusts in sand dunes (Schulz et al., 2016).

Some taxa of green algae identified in the present study have been reported from salt-affected soils. This includes taxa such as the filamentous *Dilabifilum*/*Pseudendoclonium*-like algae (Vinogradova and Darienko, 2008), *Tetracystis* (Kirkwood and Henley, 2006; Vinogradova and Darienko, 2008), *Chlorella* (Shubert and Starks, 1980; Vinogradova and Darienko, 2008) and *Borodin ellipsis* as well as *Diplosphaera chodatii*, *Chloroidium ellipsoideum* and *Stichococcus bacillaris* (Vinogradova and Darienko, 2008). Some of these algae are known to produce osmotically active substances such as sucrose (*Chlorella vulgaris,*

Chloroidium ellipsoideum), ribitol (*Chloroidium ellipsoideum*) and sorbitol (*Stichococcus* sp.), which indicates their adaption to osmotic stress (Darienko et al., 2010). In conclusion, we found several species that are typical of salt-affected environments, which supports our hypothesis that microalgae in biocrusts of potash tailings piles tolerate high salinities.

The genus *Klebsormidium* was present in none of the studied samples. In general, *Klebsormidium* is a common member of biocrusts worldwide (Glaser et al., 2018; Samolov et al., 2019). *Klebsormidium* species tolerate desiccation (Holzinger et al., 2011; Karsten et al., 2014a,b) but only moderate osmotic stress (Karsten and Rindi, 2010; Kaplan et al., 2012). *Klebsormidium* sp. cultured in 30 S_A showed a drastically reduced growth rate than in lower salinities (Karsten and Rindi, 2010). Therefore, members of this genus might not tolerate hypersaline conditions. Further, *Klebsormidium* often is observed under low pH soil conditions (Lukešová, 2001; Škaloud et al., 2014), and the neutral or slightly alkaline soil pH_{CaCl2} of the potash tailings pile sites did not provide favorable conditions. Taken together, the high soil salinity together with the measured soil pH_{CaCl2} could explain the absence of the otherwise cosmopolitan *Klebsormidium*.

In extremely saline ecosystems such as salt lakes and salt flats, *Dunaliella* is the only chlorophyte genus found that can tolerate salinities up to 170 S_A (DasSarma and Arora, 2001). This taxon was also described in soil samples (Arif, 1992; Kirkwood and Henley, 2006; Buchheim et al., 2010). In this study, *Dunaliella* was observed in brine drains at one potash tailings pile site (unpublished data). However, this genus was absent in both biocrust types, which may indicate that it is unlikely to live in the biocrust community.

Several algae occurred mainly in one crust type, in either the young or established biocrusts. For example, the filamentous cyanobacterium *Nostoc* sp. occurred in most of the samples from established biocrusts and was absent in young biocrusts. Generally, the presence of *Nostoc* sp. in the highly saline potash tailings pile areas is not surprising since it has been recorded from several salt affected habitats (Polderman, 1974; Marshall, 1982; Arif, 1992; Tsujimura et al., 1998). Tsujimura et al. (1998) reported that the abundance of Nostocales taxa was not negatively affected by soil EC, even in higher ranges. Therefore, the higher median EC_{Sat} of young biocrust soil samples cannot explain the absence of *Nostoc* sp. In contrast, filamentous *Ulothrix* or *Ulothrix-like* algae were clearly more abundant in young biocrusts, as detected by direct microscopy. Previous studies have also reported *Ulothrix* in initial biocrust stages (Zhao et al., 2008; Gypser et al., 2016). Many species of this genus are freshwater algae, whereas *Ulothrix tenerrima* was recorded in saline lakes in China (Wen et al., 2005) and *U. subflaccida* and *U. pseudoflaccida* were found in salt marshes (Polderman, 1974). The soil-stabilizing effect of the filamentous green alga *Ulothrix* in young biocrusts could be functionally replaced by filamentous cyanobacteria such as *Nostoc* sp. in established biocrusts. One may speculate that the carbon input is higher in mature biocrusts than in younger biocrusts. Therefore, in order to maintain the C:N homeostasis, N could become the limiting nutrient for organism growth. This in turn would strengthen the niche for N-fixing organisms such as *Nostoc*.

CONCLUSION AND OUTLOOK

Biocrusts occured under the extremely saline soil conditions of potash tailings pile areas and contained a diverse microalgal community including green algae, cyanobacteria and diatoms.

We identified several taxa that have been described previously in saline environments. Thus, our hypothesis that biocrusts from potash tailings piles would contain typical species of salt-affected habitats was confirmed. Further, the isolate composition (culture-dependent approach) differed between young and established biocrusts. The former showed a higher estimated abundance (culture-independent approach) of filamentous green algae such as *Ulothrix* whereas the latter exhibited a higher estimated abundance of filamentous cyanobacteria. We conclude that the occurrence of filamentous organisms is crucial for the establishment of a biocrust, but the taxa that provide this trait vary depending on the biocrust type. Our hypothesis that the number of microalgal and cyanobacterial species would be lower in young biocrusts was not confirmed, underlining the existence of a diverse algal community, even in early successional stages. pH and soil salinity, in particular Na^+ and Cl^-, affected algal isolate composition. Especially the latter effect is not surprising since potash tailings pile areas are shaped by the salt output of the salty dump material, which also results in communities of halophilic vascular plants.

Morphological species identification provided a first idea of the algal isolate composition in these extremely saline environments, but the results should be better supported by using a polyphasic approach in the future. Nevertheless, with this approach we were able to establish several clonal cultures, which could be used for greening the potash tailings piles.

Since potash tailings piles pose an ongoing environmental problem that alters the adjacent ecosystems, a vegetation cover of the pile body would be appropriate to trap rainwater, thus preventing uncontrolled leakage of the highly saline brine. However, vascular plants as a natural cover are difficult to install, as they often have deep roots and thus need a thick layer of substrate, which easily erodes on the steep flanks of the tailings pile. Biocrusts, in contrast, would only establish in the upper millimeters, which indicates the potential of biocrusts to have a positive effect on rain-water trapping. Therefore, microalgal isolates derived from biocrusts of potash tailings pile areas could offer an alternative solution for the envisaged vegetation cover. *Ulothrix* is of particular interest in this regard, since this genus was growing in young biocrusts at or very close to the potash tailings piles. The high diversity of algae in biocrusts existing under extreme saline conditions is a promising finding for the idea of greening potash tailings piles. Further studies should evaluate the potential for establishing artificial biocrusts on this extremely saline mining-waste material.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

UK and KG developed the idea. VS collected the data that was further analyzed by KG and VS. VS wrote the first draft of manuscript which was edited by UK and KG.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.00046/full#supplementary-material

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Repeated Colonization Between Arid and Seasonal Wet Habitats, Frequent Transition Among Substrate Preferences, and Chemical Diversity in Western Australian *Xanthoparmelia* Lichens

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Arid soil habitats are challenging for sedentary and slow-growing lichens because the integrity of the substrate can easily be disturbed by natural forces, e.g., wind and flood. Yet, adaptation into such habitat types occurred multiple times in lichens that may be associated with specific morphological and ecological adaptations. We studied the genetic and chemical diversity of the lichen-forming fungal genus *Xanthoparmelia* in Western Australia, where it is abundant in both arid and temperate ecoregions occurring on both soil and rock substrates. We found frequent evolutionary transitions among substrate types and between arid and temperate habitats. However, specific chemical phenotypes were not associated with different habitat and substrate types, and the level of phenotypic (the composition of secondary metabolites) divergence was not correlated with the level of genetic divergence among taxa. The study closes by discussing the importance of arid soil habitats for evolutionary diversification in the hyperdiverse genus *Xanthoparmelia*.

Keywords: Anthropocene, soil habitat, rapid diversification, repeated evolution, thin-layer chromatography

INTRODUCTION

Soil habitat is easily disturbed by natural forces, e.g., wind and flood, and anthropogenic exploitations, e.g., farming and grazing. The projected rapid and drastic changes in climate and environmental condition in the Anthropocene further imply high frequency and magnitude of disturbance of soil habitat in the near future (Zak et al., 2003; Coyle et al., 2017). Such disturbance makes soil habitat especially challenging for sedentary organisms (Coyle et al., 2017). Yet, sedentary organisms can be extremely diverse in soil habitat that might be results of high species turnover rate because of constant disturbance (Zak et al., 2003). Comparing to other types of biomes, the biodiversity of soil habitat, and especially arid soil habitat, has received much less attention

(Coyle et al., 2017). The biodiversity of arid soil habitat is, however, among the most unique and threatened ecosystems on earth in Anthropocene.

The evolution of unique phenotypes and high species diversity in soil habitat is evident in the lichen-forming fungal genus *Xanthoparmelia* (**Figure 1**; Esslinger, 1977; Elix et al., 1986; Hale, 1990; Nash et al., 1995; Blanco et al., 2004; Leavitt et al., 2018). *Xanthoparmelia* is the most species-rich genus in lichen-forming fungi (ca. 800 species; Jaklitsch et al., 2016) and is characterized by extremely high chemical diversity in terms of secondary metabolites (Elix et al., 1986; Thell et al., 2012; Divakar et al., 2013; Barcenas-Peña et al., 2018). The evolution of diverse secondary metabolites may be associated with changing habitat and substrate preferences (Boustie and Grube, 2005; Zraik et al., 2018), and has been a key diagnostic phenotypic character when identifying lichen species. In case of *Xanthoparmelia*, for example, the high species diversity and diverse secondary metabolite compositions may reflect the fact that this lineage of lichen-forming fungi have successfully adapted into and utilized a variety of habitats and substrates.

The centers of *Xanthoparmelia* species diversity are found in temperate to arid regions predominantly with Mediterranean climate (winter rain) in South Africa and Australia and each center harbors approximately 300 named species (Esslinger, 1977; Hale, 1985, 1988, 1990; Elix et al., 1986). Although *Xanthoparmelia* species in South Africa and Australia are about equally species-rich, the macro-evolutionary patterns of lineage diversification from the two geographic regions are quite distinct (Leavitt et al., 2018). There were multiple ancient lineages diversified in South Africa, while the species diversity in Australia might be a result of more recent radiation events. The data suggest that the genus originated in Gondwana and that the northern Hemisphere was colonized recently. Specifically, South Africa and nearby regions were hypothesized as the possible origin of the genus *Xanthoparmelia*, with several migrations of clades into Australia.

The typical habitats of *Xanthoparmelia* are characterized by arid to semi-arid climate with widespread open rock formation and soil substrate (e.g., **Figure 1**). Many *Xanthoparmelia* species in arid region of South Africa and Western Australia grow on soil substrate, while others in the same region grow on rocks, which is the common substrate for *Xanthoparmelia* lichens from other geographic regions (e.g., Asia, the Americas, and Europe). The soil inhabiting *Xanthoparmelia* species may exhibit specific phenotypes to grow and persist on soft and unstable substrate, such as becoming vagrant in dry season (e.g., X. hueana and X. semiviridis; Büdel and Wessels, 1986; Lumbsch and Kothe, 1988; Leavitt et al., 2011a,b). Those that occur on siliceous rock on the other hand are often attach tightly to the substrate (**Figure 1**). Some species from inselberg habitat may prefer close association with moss substrate (**Figure 1**) and become easily loose from the rock surface.

We collected samples from different ecosystems in Western Australia and obtained additional herbarium samples from Africa and other parts of Australia to study the evolutionary diversification of *Xanthoparmelia* lichen with a particular focus on the association with changes in habitat and substrate types. Note that we are interested in the changes and diversification of habitat and substrate preferences through time and their association with the evolution of different compositions of secondary metabolites. Our strategy was to sample, when possible, multiple individuals per species and to study all available chemical substances that show not only macroevolutionary (interspecific), but also microevolutionary (intraspecific) variations. Specifically, we focused on testing (1) the association between phylogenetic relatedness and habitat/substrate preferences, (2) the difference in secondary metabolite compositions and their relationship to different habitat/substrate preferences, and (3) the relationship between phylogenetic distance and chemical phenotypic dissimilarity (secondary metabolites). We estimated the frequency of evolutionary transition between different habitat/substrate preferences and if specific chemical phenotypes have evolved to adapt to the different preferences. This study was finalized by discussing the importance of arid soil habitat on promoting and maintaining high species diversity for *Xanthoparmelia* lichens.

MATERIALS AND METHODS

Sampling, Chemical Profiling, and DNA Extraction

The Western Australian *Xanthoparmelia* samples were collected during a field trip in January 2018. Additional samples from Eastern Australia and Africa were obtained through loans from the Australian National Herbarium (CANB), the East African Herbarium of National Museum of Kenya (EA), and the Pharmacy Herbarium of the Complutense University of Madrid (MAF). We soaked the samples (approximately 0.2 g of thallus fragments per specimen) in acetone overnight to remove secondary metabolites before DNA extractions. The acetone extract was used for identification of the secondary metabolites using high-performance thin layer chromatography (HPTLC), using solvent system C following established methods (Culberson and Johnson, 1982; Arup et al., 1993; Lumbsch, 2002; Orange et al., 2010). Note that the presence and absence calling for each secondary metabolite were done by the same person to ensure a consistent result reading among HPTLC plates. We subsequently extracted total genomic DNA from the thallus fragments (after removing the acetone) using the ZR Fungal/Bacterial DNA Miniprep Kit (Zymo Research Corp., Irvine, CA, United States) following the manufacturer's instructions.

DNA Sequence Processing and Phylogenetic Reconstruction

We amplified the internal transcribed spacer (ITS) region using a combination of the primers ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990). PCR reactions contained 6.25 μl of MyTaq Mix, 5.25 μl H_20, 0.25 μl forward and reverse primers, and 0.5 μl template DNA for a total of 12.5 μl. PCR products were sequenced using an ABI 3730 DNA Analyser (Applied Biosystems) at the Pritzker Laboratory

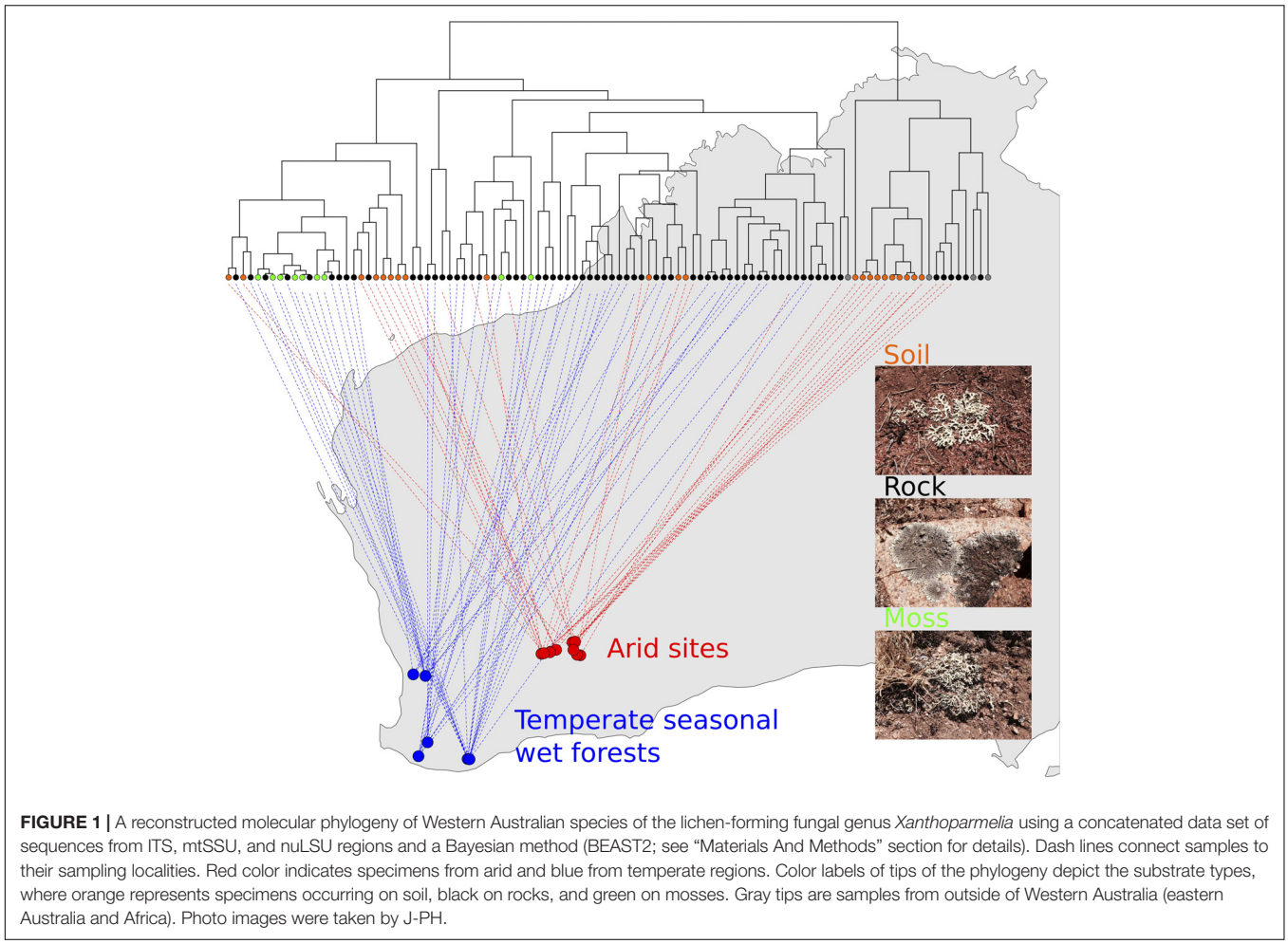

FIGURE 1 | A reconstructed molecular phylogeny of Western Australian species of the lichen-forming fungal genus *Xanthoparmelia* using a concatenated data set of sequences from ITS, mtSSU, and nuLSU regions and a Bayesian method (BEAST2; see "Materials And Methods" section for details). Dash lines connect samples to their sampling localities. Red color indicates specimens from arid and blue from temperate regions. Color labels of tips of the phylogeny depict the substrate types, where orange represents specimens occurring on soil, black on rocks, and green on mosses. Gray tips are samples from outside of Western Australia (eastern Australia and Africa). Photo images were taken by J-PH.

for Molecular Systematics and Evolution at the Field Museum, Chicago, IL, United States. The sequencing results, quality check, and sequence alignment were done using Geneious v11.0.3[1] (Kearse et al., 2012). We subsequently used the automatic option in MAFFT v.7 (Katoh and Standley, 2013) to realign the sequence alignment output from Geneious. A maximum likelihood phylogeny of the ITS sequences was reconstructed using PhyML (Guindon et al., 2010) with smart model selection (Lefort et al., 2017) and 100 bootstrapping for evaluating branching supports.

We further sequenced two additional loci (mitochondrial small subunit rDNA [mtSSU] and nuclear large subunit rDNA [nuLSU]) by PCRs and Sanger sequencing or by extracting the sequence data from existing metagenomic data sets (following Barcenas-Peña et al., 2018). The primer sets for mtSSU (mrSSU1 and mrSSU3; Zoller et al., 1999) and nuLSU (LR0R and LR6; Vilgalys and Hester, 1990), were used to amplify the two additional loci. The PCR reactions and conditions as well as DNA sequencing procedure were the same as those for the ITS region. The newly obtained mtSSU and nuLSU sequences were subsequently aligned separately using the automatic option in MAFFT v.7. We did not perform ML phylogenetic analysis for nuLSU and mtSSU separately; instead we used nuLSU and mtSSU sequences for a Bayesian phylogenetic analysis. Specifically, we aimed to examine whether the inclusion of additional loci will alter the reconstructed phylogenetic relationships, increase the robustness of nodal supports, and affect the resulted evolutionary inferences. A concatenated data set, where each of the three loci (ITS, mtSSU, and nuLSU) was aligned separately, was imported into BEAST 2 with BEAGLE phylogenetic library for phylogenetic reconstruction (BEAST2 ver. 2.6; Bouckaert et al., 2019; BEAGLE3; Ayres et al., 2019). Each locus was given its own transition rate and clock models, while the tree topology was specified as linked across loci. We selected the GTR + I + Gamma model for all three loci allowing the Bayesian approach to search all possible parameter spaces without the unnecessary tests for best-fit molecular evolution model (as suggested by Nascimento et al., 2017). The MCMC search was run for 10^{10} generations with parameters and trees samples every 10^5 generations. The MCMC search result was imported into tracer (ver. 1.7; Rambaut et al., 2018) for estimating the effective sample size (ESS) for each parameter estimation and to access the convergence of Bayesian analysis. After discarded the first 20% trees as burnin, the remaining post-burnin trees (800000 trees) were imported

[1]http://www.geneious.com

into treeAnnotator (a companion program with BEAST2) to build a maximum clade credibility (MCC) tree.

Estimating Transitions Between States of Geographic Origin and Among States of Substrate Preference

The geographical and substrate states were coded as binary (0 and 1 for arid/semi-arid and temperate regions, respectively) and three discrete states (0, 1, and 2 for rock, soil, and mosses, respectively) (**Supplementary Files: state_sort.txt and state_sort2.txt**). We used the ace function in the R package ape (Paradis and Schliep, 2019) to estimate the transition rates between character states and to estimate the ancestral states on nodes of the phylogeny. Specifically, we calculated the likelihoods for models assuming equal/symmetric (ER/SYM) or asymmetric (ARD) transition rates between states and then used likelihood ratio test to determine the best model for the data sets. The ancestral state on the nodes was then estimated based on the best model. We used both the reconstructed Bayesian phylogenetic tree based on the concatenated data set and the ML reconstruction of ITS only phylogeny to estimate the transitions between states and ancestral states.

Assessing Chemical Diversity and Correlation Between Phylogenetic and Chemical Divergence

We used non-metric multidimensional scaling (NMDS) implemented as the metaMDS function in the R vegan package (Dixon, 2003) to study chemical diversity in Western Australian *Xanthoparmelia* species. A matrix of presence (1) and absence (0) for all the identified secondary metabolites (see "Results" section) was used to calculate the Euclidean distances between pairs of samples (k = 2). We subsequently used the ordiplot function to visualized the distribution of samples in a two dimensional space. We further investigated the correlation between distance calculated from multidimensional space and distance estimated from k = 2 using the stressplot function. Specifically, if a strong correlation is found between the distance matrices, then it implies that out two dimensional plot from NMDS analysis is a good representation of the multidimensional data set. To statistically evaluate whether lichen samples from different geographic regions or that have different substrate preferences may exhibit different secondary metabolites, we used a permutational multivariate analysis of variance using distance matrices with 1000 permutations implemented as the adonis function in the R package vegan.

The phylomorphospace function implemented in phytools (Revell, 2012) was utilized to visualize correlation between phylogenetic distance and chemical difference in secondary metabolite. Specifically, the x and y coordinates from NMDS analysis were used as phenotypic data sets for phylomorphospace plot. Furthermore, we statistically tested whether a significant correlation between phylogenetic distance and chemical difference can be found in our data set. Phylogenetic distance between pairs of samples was calculated based on both the concatenated Bayesian phylogeny and the reconstructed ITS ML phylogeny using the cophenetic function implemented in ape. The difference in secondary metabolites between pairs of samples was represented by Euclidean distance calculated based on the x and y coordinates from NMDS analysis. We also used the phylosig function implemented in phytools to test for phylogenetic signal (method = k; Blomberg et al., 2003) using the coordinates from the first and second axes of the NMDS results. The significance of phylogenetic signal was evaluated using 1000 simulations in randomization test.

RESULTS

Rampant Transitions Between Geographic Origins and Among Substrate Preferences

A total of 104 ITS sequences were newly generated from 88, 12, 2, and 2 specimens from Western Australia, other parts of Australia, Kenya, and Namibia, respectively (GenBank accession numbers: MT94747 – MT948500). An alignment of 484 base pairs was produced after sequence processing (available as a **Supplementary Material: ITS.nex**). The reconstructed ITS ML phylogeny of our *Xanthoparmelia* samples revealed a pattern of rampant transitions between arid and temperate regions and among different states of substrate preference (**Supplementary: ITS_FigS1.pdf**). The support values for the clades, however, were low in most cases (<50%; **Supplementary File: PhyML.tre**).

A total of 49 and 73 mtSSU and nuLSU sequences were obtained from samples from Western Australia (GenBank accession numbers: MT93681 – MT93729 and MT93267 – MT93339 for mtSSU and nuLSU, respectively; **Supplementary: DNA_sequence_result.xlsx in**). Alignments of 917 and 974 base pairs were resulted after sequence processing for mtSSU and nuLSU loci, respectively; a concatenated sequence file was produced that included all three sequenced loci (**Supplementary: concatenated_3loci.nex**). All parameters in the BEAST2 analysis had >250 ESSs. The likelihood of the tree topology search reached a plateau after generation 1.8×10^9. The reconstructed Bayesian phylogeny also revealed a pattern of rampant transitions between arid and temperate regions and among different states of substrate preference (**Figure 1**). The posterior supports for many nodes, particularly those pertaining to deep branches, were low (<50%; **Supplementary: MCC_tree.pdf and MCC_tree.tre**).

Our ML ancestral state estimation based on the Bayesian phylogeny using the three loci concatenated data set (**Figure 1**) indicated a symmetric transition rate between geographic states and the ancestral states at deeper nodes of the phylogeny were highly uncertain (**Figure 2**). We found the same results of high uncertainty when estimating the ancestral state of substrate preference and high transition rates among states (**Figure 2**). The transition rates among substrate states were asymmetric; the transition from rock to soil and from rock to moss were higher

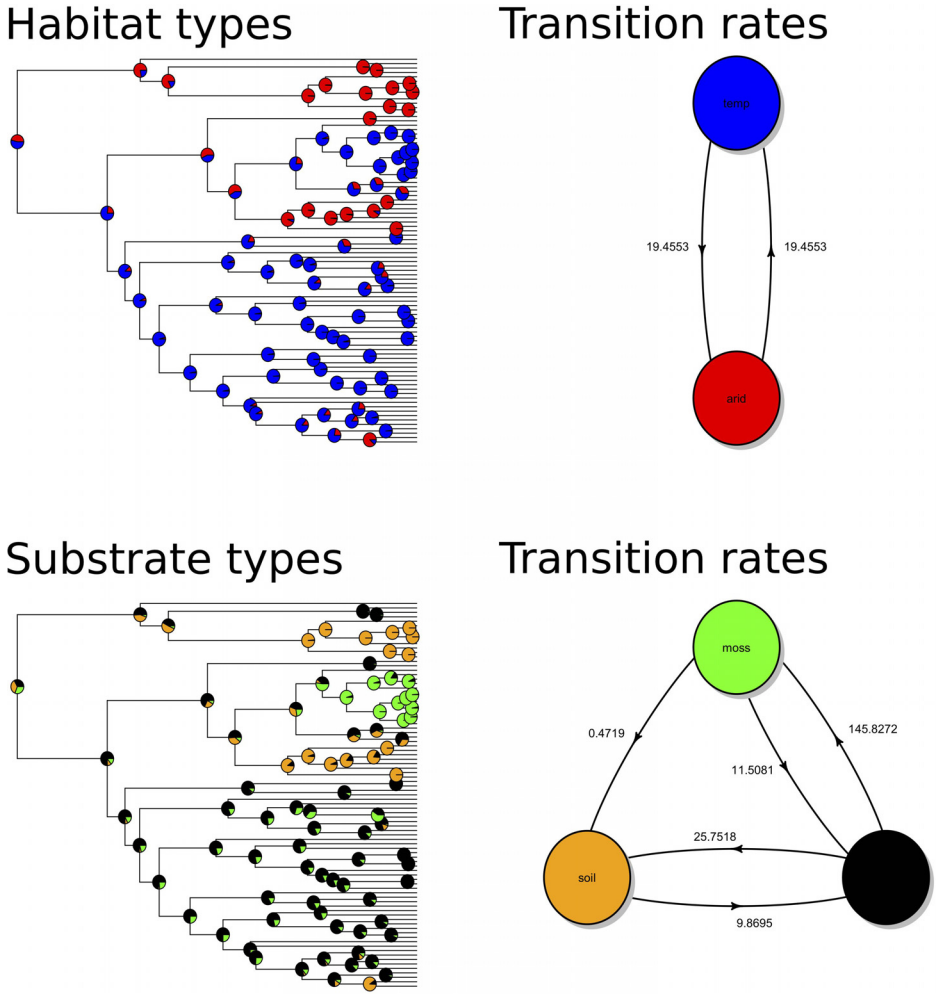

FIGURE 2 | Ancestral state estimations of habitat (upper panels) and substrate (lower panels) types and the corresponding maximum likelihood estimates of transition rates between states.

than the reverse directions (**Figure 2**). The analyses using ML phylogeny from ITS only data set resulted in the same inferences (**Supplementary: ITS_FigS2.pdf**).

Lack of Correlation Between Chemical Phenotypes and Habitat/Substrate Types

Our chemical profiling tests using HPTLC identified a total of 25 secondary metabolites (**Supplementary: Chemistry.xlsx**), of which 24 were variable among samples (**Figure 3**). Using presence and absence data matrix of secondary metabolites (those that have trace amount were coded as present), our NMDS result showed that the distribution of secondary metabolite diversity overlapped among samples from different geographic regions (arid versus temperate) and those that have different substrate preferences (rock, soil, and moss) (**Figure 4**). The stressplot showed a high level of correlation between observed dissimilarity and ordination distance (**Supplementary: stressplot.pdf**). Our permutational multivariate analysis also indicated statistically non-significant different secondary metabolite compositions among samples between geographical regions and among samples occurring on different substrates.

Phylogenetic Divergence Does Not Predict Phenotypic Difference

Our phylomorphospace plot based on the Bayesian phylogeny (**Figure 1**) and the NMDS results (**Figure 4**) showed that distantly related samples can exhibit very similar chemical properties and that closely related samples may have distinct compositions of secondary metabolites (**Figure 5**). Similarly, we did not find a significant correlation between phenotypic divergence and phylogenetic distance (**Figure 5**). Furthermore, our test for phylogenetic signal using coordinate data as continuous variable were statistically non-significant ($P > 0.05$ in both cases using NMDS1 and NMDS2 axes). The analyses using ML phylogeny from ITS only data set also resulted in the same inferences (**Supplementary: ITS_FigS5.pdf**).

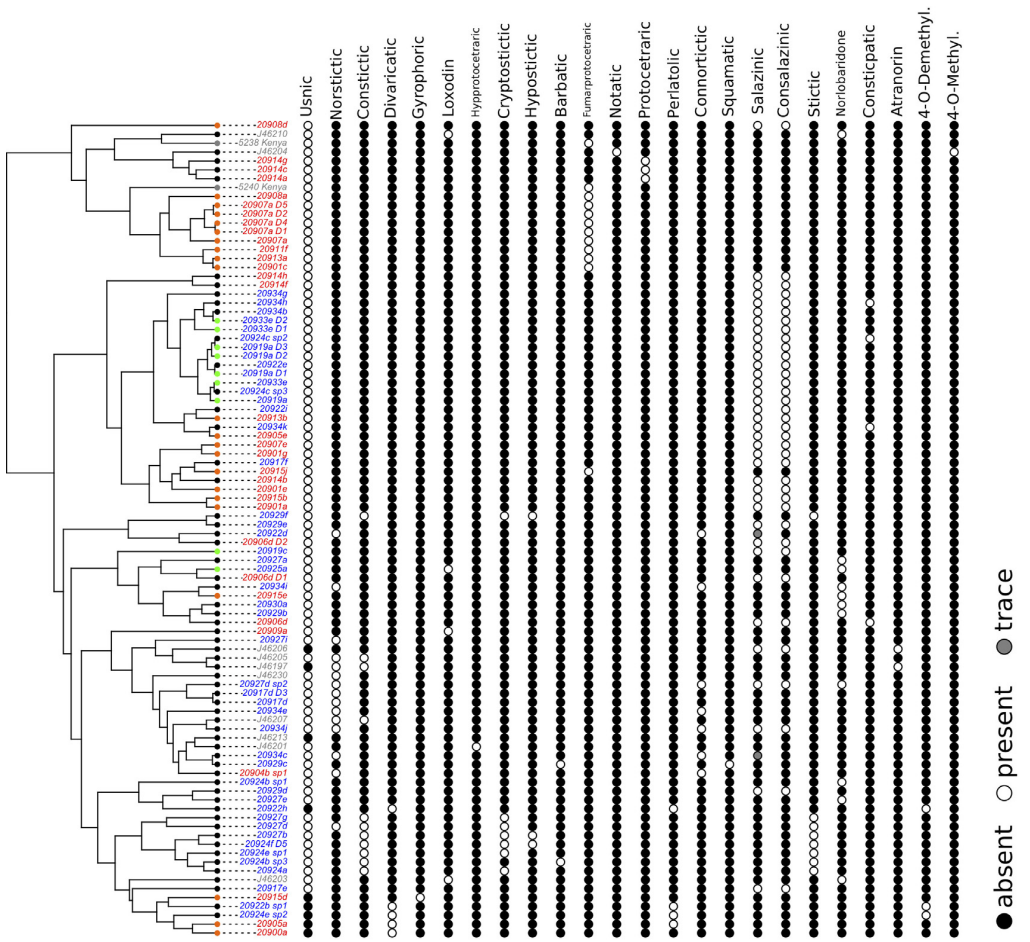

FIGURE 3 | The presence and absence of 24 tested chemical substances for each examined sample in our study (one of the substance was invariably absent in all the samples and was not shown in the figure – succinprotocetraric). Different colors of the tips of the phylogeny depict their substrate types, while colors of the specimen names depict the geographic origins (see **Figures 1**, **2** for details).

DISCUSSION

The results from this study demonstrated that *Xanthoparmelia* lichens exhibit high genetic and phenotypic diversities across the temperate and arid ecoregions and between soil and rock substrate types in West Australia. As expected for a lineage that underwent recent rapid diversification (Leavitt et al., 2018), our results revealed that many phylogenetic relationships in our reconstructed phylogenies are not well-supported. The inferences made from our phylogenetic comparative study may thus be compromised because different inferences may be made should different branching topologies were provided. We would like to emphasize that, however, when different tree topologies were utilized in our analyses, i.e., a Bayesian phylogeny using a concatenated data set of three loci and a ML phylogeny using ITS sequences, our resulted inferences about the evolutionary history were consistent. We showed that *Xanthoparmelia* species have repeatedly colonized and rapidly diversified in arid soil habitats even though soil habitat can be easily disturbed by both natural and artificial forces and presents a challenging environment to sedentary organisms. The arid soil habitats harbor unique *Xanthoparmelia* lineages, where their close relatives can be found on nearby rock substrate or in the temperate region. However, we did not find evidence for specific secondary metabolite compositions in different ecoregions nor for different substrates. Furthermore, we found that the dissimilarity in secondary metabolites between pairs of samples did not correlate with phylogenetic distance implying swift phenotypic transitions.

A number of lichen species exhibit large distributional ranges, often being cosmopolitan, pantropical or antitropical (e.g., Galloway, 2008; Werth, 2011; Printzen et al., 2013; Leavitt and Lumbsch, 2016). However, molecular data have shown that many widely distributed species consist of separate lineages on different continents (Arguello et al., 2007; Otálora et al., 2010; Amo de Paz et al., 2012; Divakar et al., 2019). Species with worldwide distributional ranges usually are restricted to specific habitats – e.g., arctic species that occur in temperate regions are usually restricted to higher altitudes (Poelt, 1987). Studies discovering cryptic species in lichen-forming fungi mostly found distinct lineages occurring in different geographical regions,

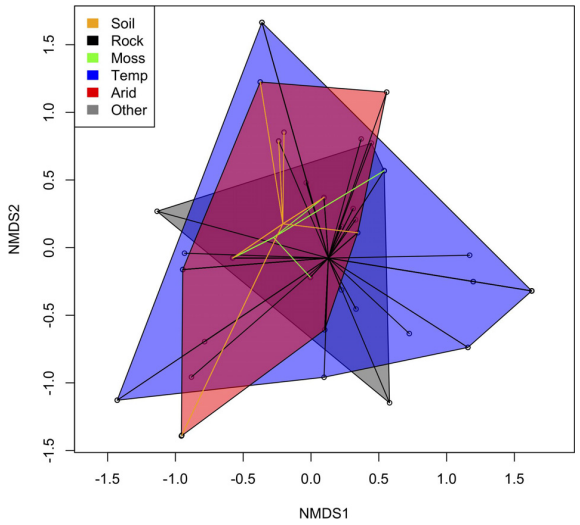

FIGURE 4 | Result from non-metric multidimensional scaling (NMDS) analysis using presence/absence data from 25 chemical substances based on high-performance thin layer chromatography (HPTLC) assays. Open dots represent studied samples. Color lines connecting dots indicate different substrates. Orange indicates specimens on soil, black on rocks, and green on mosses. Colored areas indicate geographic localities, where blue shows samples collected from temperate/Mediterranean climate region and red from arid region.

which is consistent with the competitive relatedness hypothesis that postulates that closely related species should not co-occur in the same community, since competitive interactions would lead to exclusion of less competitive species (Cahill et al., 2008). However, a few studies failed to demonstrate ecological difference of closely related cryptic lineages (Parnmen et al., 2012, 2013; Kraichak et al., 2015). In tropical foliicolous lichens the pattern of multiple closely related species co-occurring on the same leaf was explained as being a result of the highly dynamic substrate (Lücking, 2001; Lücking and Bernecker-Lücking, 2002).

This is the first study specially addressing the issue of phylogenetic relationships of co-occurring closely related lichen-forming fugal species. Our result indicate that closely related *Xanthoparmelia* can live in arid and temperate regions. It is possible that long distance dispersal, as observed in many lichen species (e.g., Fernández-Mendoza and Printzen, 2013), facilitates the colonization of the same habitat by closely related lineages across large geographic distances. However, it is also possible that the same evolutionary lineage can evolve different preferences rapidly and repeatedly. Our result using a densely sampled *Xanthoparmelia* data set in Western Australia shows that transitions between the two focal habitat types can occur rapidly and repeatedly within an evolutionary lineage (**Figures 1**, **2**). We note that temperate *Xanthoparmelia* species in Western Australia usually occur on open rock formations, e.g., inselbergs, and therefore the differences in ecological preference may not be drastic. For example, the inselbergs habitats are open habitats that are directly exposed to sunlight; additionally the substrate does not conserve water. That is, *Xanthoparmelia* lichens may still prefer arid microhabitat islands within temperate seasonal wet forests.

The frequent transitions between substrate types in *Xanthoparmelia* is surprising, because lichens often have specific substrate preferences, often invariable over evolutionary time (Brodo, 1973; Gaya et al., 2015). It has been hypothesized that it may require specific phenotypic adaptation to help with attaching to different substrate types; furthermore, different fungal lineages often have very different chemistry preferences (e.g., pH value) of their substrate (Brodo, 1973). *Xanthoparmelia* species generally associated with siliceous rock or sandstone worldwide (Hale, 1985, 1988, 1990; Elix et al., 1986; Barcenas-Peña et al., 2018; Leavitt et al., 2018). We found samples from different substrate types having overlapped distribution of secondary metabolite compositions (**Figure 4**), which supports the hypothesis that rock and soil substrate types may exhibit similar chemical profiles. Furthermore, when carefully examining the profiled chemical substances, we could not identify associations between the absence/presence of any secondary metabolite and specific habitat/substrate preferences (**Figure 3** and **Supplementary: ITS_FigS3.pdf**). The rapid adaptation to differences in the structure integrity between soil/moss and rock is rather puzzling and requires future studies to understand the underlying mechanisms allowing the rapid and repeated transitions. Note that the pattern of repeatedly colonizing soil habitats is not unique to Western Australian *Xanthoparmelia*. The same pattern can also be found in South African *Xanthoparmelia* species (Esslinger, 1977; Hale, 1985, 1988, 1990; Elix et al., 1986), including multiple parallel evolutions of vagrant phenotype (Leavitt et al., 2011a,b).

The genus *Xanthoparmelia* has a high chemical diversity (Hale, 1985, 1988, 1990; Elix et al., 1986). Specifically, differences in secondary metabolites have conventionally been utilized as a main phenotypic character to circumscribe species boundaries. We identified 24 chemical substances with their presence (including trace amount) and absence varying among samples (**Figure 3**), and there is a total of 28 unique chemical combinations among the 148 samples (**Supplementary file: AustraliaSpChemistry.xlsx**). With the combination of substrate type, presence/absence data from TLC analyses, spot test results, and morphological diagnosis, we have identified >60 putative species lineages in the data set (**Supplementary: Chemistry.xlsx**). Our results confirm that *Xanthoparmelia* is a species rich genus that exhibits high diversity in its chemical substances.

Our results showed that the evolution of secondary metabolite composition can be rapid (**Figures 4**, **5** and **Supplementary: ITS_FigS4.pdf and ITS_FigS5.pdf**), although samples of the same species usually have the same combination of secondary metabolites. Specifically, closely related samples may exhibit distinct chemical profiles (**Figure 5A**). Similar patterns have been reported in *Cladia aggregata* species complex (Parnmen et al., 2012) where even putative conspecific samples, intraspecific microevolutionary pattern, may exhibit different chemical profiles. By a broad sampling design across multiple distantly related lineages, our result further demonstrated that this rapid evolution of chemical phenotypes may also result in convergence between distantly related samples, i.e., macroevolutionary

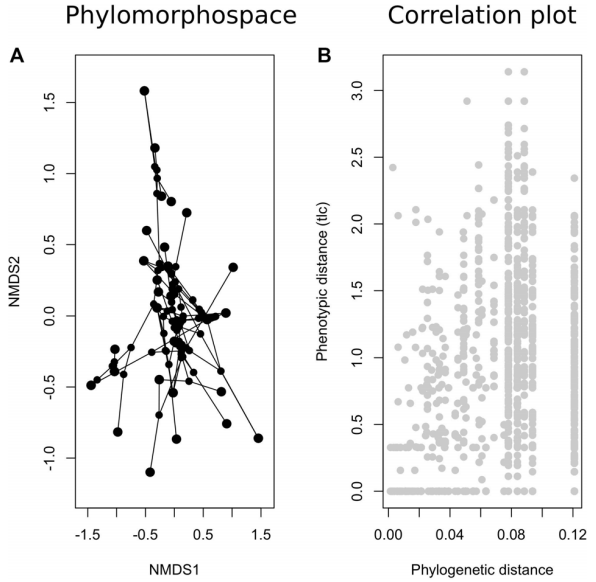

FIGURE 5 | Plots of the relationship between phenotypic divergence and phylogenetic distance. **(A)** a phylomorphospace plot; **(B)** a plot of phenotypic distance between pairs of samples based on HPTLC data set against the estimated phylogenetic distance extracted from the Bayesian phylogeny using a concatenated sequence data set of three loci (see **Figure 1**).

divergence and convergence. That is, similar chemical divergence between closely related taxa can be observed between distantly related samples as well; furthermore, there is no association found between phylogenetic distance and phenotypic dissimilarity (**Figure 5B**). It is difficult to infer why similar chemical profile emerge repeatedly among distantly related species in *Xanthoparmelia* species. However, the result of rapid divergence and convergence of chemical phenotypes along with repeated evolution of substrate preference among distantly related samples, whereas rapid divergence was observed among closely related samples as discussed above implies a recurrent pattern that *Xanthoparmelia* species may effectively and rapidly adapt to, or cope with environmental challenges.

Our result demonstrates that arid soil habitat, despite being potentially easily disturbed by natural and anthropogenic forces, is critical for *Xanthoparmelia* diversification and preserves high species as well as chemical diversity. Specifically, transitions between soil and rock habitats and between arid and temperate regions may have promoted rapid diversification among closely related lineages and facilitated local increase of species diversity. Australia was only colonized recently (<10 MYA) by a few *Xanthoparmelia* lineages (Leavitt et al., 2018), yet the species number is much higher than those from Eurasia and the Americas. Note that the colonization of the Holarctic *Xanthoparmelia* was about the same time period as the Australia *Xanthoparmelia*, and therefore the difference in species number could not be explained by this difference in the time of colonization. However, the difference could be explained in part by the observation that only one lineage has successfully colonized the northern Hemisphere, while at least three lineages have colonized Australia independently (Leavitt et al., 2018). A common feature between the two diversity hotspots of *Xanthoparmelia* – Australia and South Africa – is the availability of arid soil habitat. Future studies that focus on the evolutionary history of South African *Xanthoparmelia* species are needed to validate our inference that arid soil habitat could have been an important factor that not only promote the rapid speciation, but also the maintenance of recently diverged evolutionary lineages from merging, or competitive exclusion.

DATA AVAILABILITY STATEMENT

The sequence data have been uploaded to GenBank (accession numbers: MT93267–MT93339, MT93681–MT93729, and MT94747–MT94850).

AUTHOR CONTRIBUTIONS

J-PH and HL conceived the study and collected the lichen samples. KA, AB-P, and SK-L generated molecular data and performed TLC experiments with input from J-PH. HL and J-PH performed the analyses. All authors wrote the manuscript and read and approved the final 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/fevo.2020.00129/full#supplementary-material

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Ecophysiological Response Against Temperature in *Klebsormidium* (Streptophyta) Strains Isolated From Biological Soil Crusts of Arctic and Antarctica Indicate Survival During Global Warming

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Global warming, as global problem, may particularly affect the vegetation of the Polar Regions. Biological soil crusts (BSCs) as pioneer communities perform a variety of important ecological functions under the harsh environmental conditions at high latitudes. The green algal genus *Klebsormidium* is a common member of BSCs and in the present study, the ecophysiological resilience to temperature stress of 20 strains from Arctic and Antarctica were investigated. All 20 *Klebsormidium* strains exhibited the capability to grow under a wide temperature range (from 6 to 28°C) and hence were characterized as psychrotolerant with optimum growth temperatures between 18°C and 26°C. Statistical analyses showed no significant differences in optimum growth temperature. However, growth rates at optimal temperatures varied between strains and indicated infraspecific physiological plasticity. Furthermore, correlation with the sampling sites as well as different BSC types were examined but no significance was confirmed. Our results revealed that Polar *Klebsormidium* strains are able to survive such changing conditions, and even benefit from higher environmental temperatures.

Keywords: Aeroterrestrial algae, growth, ecophysiology, temperature stress, Polar Regions

INTRODUCTION

Biological soil crusts (BSCs) are complex agglomerations formed by diverse phototrophic and heterotrophic organisms such as microalgae, cyanobacteria, lichens, bryophytes, bacteria, microfungi and microfauna (Belnap, 2006; Darby and Neher, 2016). These pioneer communities constitute the dominant vegetation cover on the temporarily snow- and ice-free soil surfaces of the Polar Regions and perform a wealth of ecological functions (Yoshitake et al., 2010; Williams et al., 2016). BSCs stabilize soil against water and wind erosion as well as cryoturbation, contribute to primary production, carbon and nitrogen fixation and benefit the growth of vascular plants (Belnap, 2002; Eldridge and Greene, 1994; Evans and Johansen, 1999; Yoshitake et al., 2010).

The filamentous green alga genus *Klebsormidium* (Klebsormidiophyceae, Streptophyta) can be found worldwide in numerous habitats (Elster et al., 2008; Škaloud and Rindi, 2013) and is also commonly associated with BSCs in both the Arctic and Antarctica (Hayashi and Shinozaki, 2012; Pushkareva et al., 2016; Borchhardt et al., 2017a,b; Rippin et al., 2018). This genus has a pivotal role in the formation of BSCs by its filamentous morphology as well as excretion of extracellular polymeric substances (EPS), which promote the moisture retention and stabilization of the soil surface (Barberousse et al., 2006; Hu et al., 2009; Büdel et al., 2016).

The Polar Regions are characterized by harsh environmental conditions such as low temperatures, a pronounced seasonality, scarcity of liquid water and strong solar radiation, and *Klebsormidium* has the potential to cope with these stressors (Elster et al., 2008; Karsten and Rindi, 2010; Kaplan et al., 2012; Holzinger and Karsten, 2013; Kitzing et al., 2014). The ecophysiology of *Klebsormidium* has been studied on several strains from different biogeographically regions (e.g., Holzinger et al., 2014; Herburger and Holzinger, 2015; Blaas and Holzinger, 2017; Donner et al., 2017). It is known that members are poikilohydric, which describes the inability to actively regulate cellular water content. Therefore, organisms nearly completely dehydrated (Holzinger et al., 2014; Karsten and Holzinger, 2014). The electron transport chains in the thylakoid membranes are affected by cellular desiccation and inhibit photosynthesis (De Winder et al., 1990; Gray et al., 2007; Fernández-Marín et al., 2016). However, studies on Arctic and Antarctic *Klebsormidium* strains showed resistance against desiccation induced injuries and investigation on alpine strain of *Klebsormidium crenulatum* revealed molecular mechanisms such as up-regulation of photosynthetic transcripts caused by dehydration (Elster et al., 2008; Holzinger et al., 2014). Furthermore, strains accumulate osmolytes (raffinose and sucrose), free amino acids (γ-aminobutyric acid) and starch grains and oil droplets in order to retain water within the cells and protect against membrane damage and protein aggregation, which also caused by freezing (Bisson and Kirst, 1995; Manabu et al., 2008; Holzinger et al., 2014). However, the knowledge on the capabilities of Polar *Klebsormidium* strains to cope with increasing temperatures due to the climate change is insufficient.

Climate change is already happening. An increase of annual mean temperature of more than 1°C was determined in the Polar Regions in the last decades and the atmospheric temperature in the Artic has doubled since 1980 (Kejna et al., 2013). Mean air temperature of Antarctic King George Island was −4.3°C in 1959, while only −1.1°C was measured in 2008 (Kejna et al., 2013). It is presumed that the BSCs of the Polar Regions cannot resist the rapid climate change, and hence their composition as well as distribution will shift or BSCs will even be displaced by invasive species (Frenot et al., 2005; Pushkareva et al., 2016). Previous results of a pedological analysis of polar BSC species showed no significant influences of C, N, S, TP (total phosphorus) and pH. In addition, a statistically significant influence of precipitation on species composition was determined (Borchhardt et al., 2017b). Therefore, studies on ecophysiological performance of Polar strains not only merit a particular attention but also urgently needed. Findings, whether and how Artic and Antarctic *Klebsormidium* strains cope with higher temperatures, might enable to predict the development of this key vegetation in the Polar Regions under the climate change scenarios.

MATERIALS AND METHODS

Study Sites

Biological soil crusts samples were collected at Svalbard (Arctic) and King George Island as well as Ardley Island (South Shetland Islands, Antarctic Peninsula, **Figure 1** and **Table 1**). Crust material was cut out of the soil surface using plastic petri dishes (60 mm Ø) and a spatula. BSCs were air dried with open lids to avoid mold growth and stored in paper bags at a dark place.

The archipelago Svalbard located in the Arctic Ocean ranges from 74 to 81° north latitude and from 10 to 35° east longitude. Svalbard has a mild climate compared to regions at the same latitudes because of the West Spitsbergen Current (WSC), which transports warm Atlantic water masses into the Arctic Ocean along the West coast. The mean temperature in summer is 7 and −14°C (minimum −35°C) in winter (Norwegian Meteorological Institute)[1].

King George Island and the nearby Island Ardley belong to the South Shetland Islands, which are located in maritime Antarctica (61 to 63° south latitude, 54 to 63° west longitude). The archipelago is separated from the Antarctic Peninsula by the Bransfield Strait and from South America by the Drake Passage. Because of the Antarctic Circumpolar Current (ACC) the maritime Antarctica has milder climate with mean annual temperature of −2.5°C on King George (Kejna et al., 2013).

Algal Isolates and Cultivation

Biological soil crusts samples were cultured on 1.5% Difco™ Agar (Becton Dickonson GmbH, Heidelberg, Germany) enriched with Bold's basal medium with vitamins (Starr and Zeikus, 1993) modified by tripled nitrate concentration (3N-MBBM + V). To obtain unialgal isolates enrichment cultures were regularly screened for colonies using stereo microscope (ZS40, Olympus, Tokyo, Japan) with a magnification of 40-fold. Potential microalgal colonies were isolated from the enrichment cultures with a needle and transferred to a new agar plate. The growth of colonies was frequently monitored with the stereo microscope and several sub-isolates were generated for further purification. If no contamination with concomitant microalgae or fungi was detected the isolate was transferred to a new agar plate under sterile conditions. Strains were identified using light microscope (BX-51, Olympus, Tokyo, Japan) following the identification key of Ettl and Gärtner (2014) and descriptions by Rindi et al. (2011) as well as Mikhailyuk et al. (2014). Nine Antarctic Klebsormidium strains and eleven Arctic strains were isolated and part of the Culture Collection at the University of Rostock. One strain (CCALA 708) was delivered from the Culture Collection of Autotrophic

[1]https://www.met.no

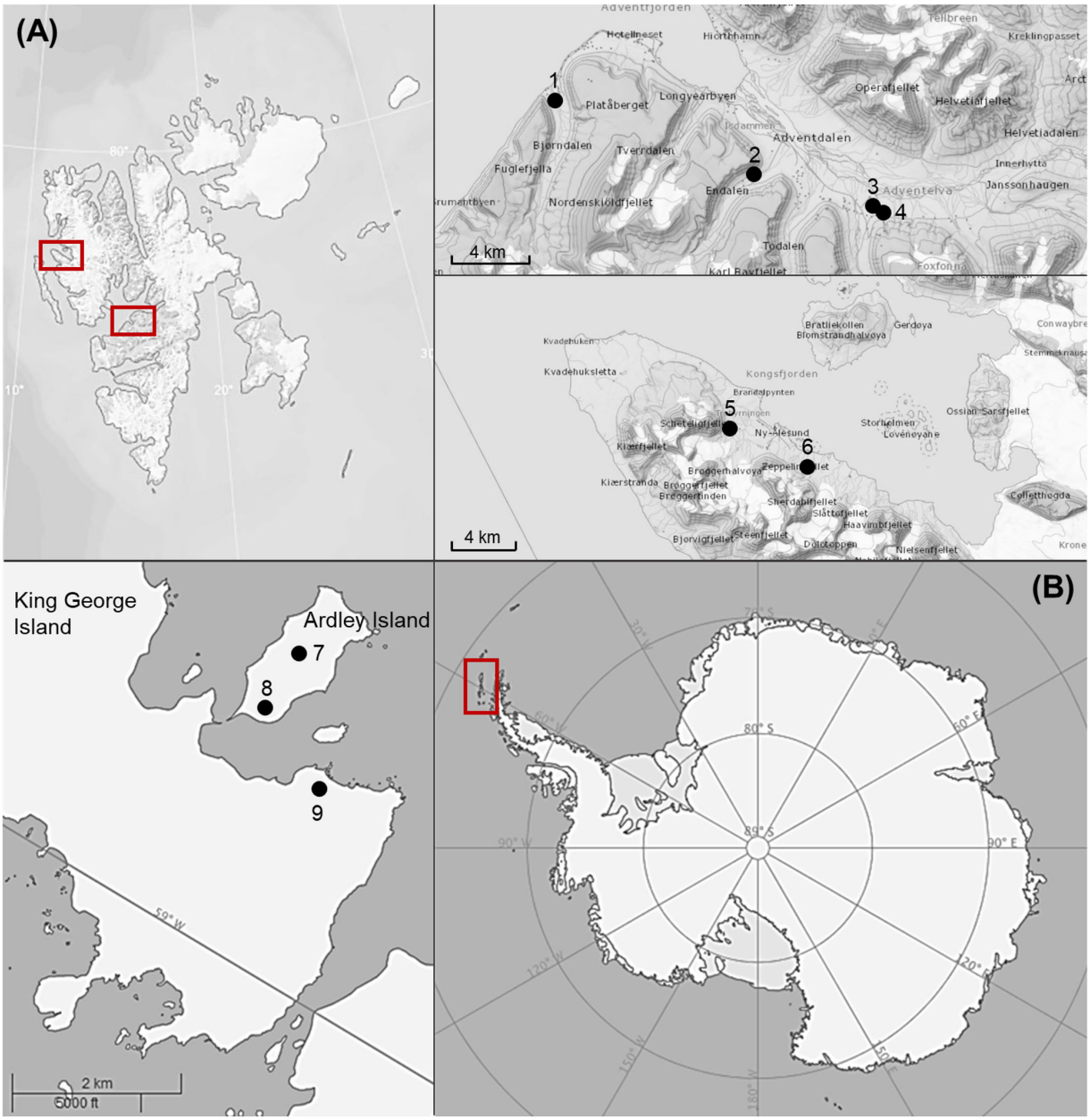

FIGURE 1 | Map of sampling areas for collected biological soil crusts. **(A)** The two investigated sampling localities on Arctic Svalbard (around Ny-Ålesund and Longyearbyen), **(B)** The two investigated sampling localities on Antarctic South Shetland Islands (Ardley and King George Island). Numbers of sampling stations are explained in **Table 1**.

Organisms (CCALA, Institute of Botany, Charles University, Tøeboò, Czechia).

Isolates were cultured at 15°C and 35 µmol photons m-2 s-1 (Daylight Lumilux Cool White lamps L36W/840, OSRAM Licht AG, Munich, Germany) under a 16:8 h light-dark cycle.

Growth in Response to Temperature

For *in vivo* measurement of the effect of 12 different temperatures on growth, as temperature gradient from 6, 8, 10, 12, 13, 14, 17, 18, 20, 23, 26 to 28°C, chlorophyll *a* fluorescence was used as a proxy for biomass accumulation according to Gustavs et al. (2009). Growth experiments were carried out using a modified self-constructed algal incubator (Kunststoff-Technik GmbH, Rostock, Germany) as described in Woelfel et al. (2014). with the possibility to create a temperature gradient at 35 µmol photons m-2 s-1 (LED) under a 16:8 light-dark cycle. All measurements were always done with log-phase cultures, which were pre-incubated at experimental conditions for 10 days. Pre-cultures were inoculated with 100 µl of liquid stock culture and were kept on 24 well plates. Each well was filled with 2.5 ml

TABLE 1 | Overview of used *Klebsormidium* strains including sampling stations on Arctic Svalbard and Antarctic King George and Ardley Island.

Strain	Species	Station number	Coordinates
Björn1C	*Klebsormidium* cf. *subtile*	1 (Björndalen)	78°13.167″N 15°18.777″E
Björn-11a	*Klebsormidium* cf. *crenulatum*	1 (Björndalen)	78°13.167″N 15°18.777″E
EiE1B	*Klebsormidium* cf. *subtile*	2 (Eindalen entrance)	78°11.180″N 15°45.662″E
EiE-14a	*Klebsormidium* cf. *Subtile*	2 (Eindalen entrance)	78°11.180″N 15°45.662″E
EiE-15a	*Klebsormidium* cf. *Dissectum*	2 (Eindalen entrance)	78°11.180″N 15°45.662″E
ADC1H	*Klebsormidium* cf. *klebsii*	3 (Adventsdalen, camp)	78°10.292″N 16°00.574″'E
AD-14a	*Klebsormidium* cf. *subtile*	4 (Adventsdalen)	78°10.205″N 16°01.336″E
AD-16a	*Klebsormidium* cf. *nitens*	4 (Adventsdalen)	78°10.205″N 16°01.336″E
CCALA708	*Klebsormidium* cf. *montanum*	5 (subglacial)	78(°55.480(N 11°46.408″E
Hinter-11all	*Klebsormidium* cf. *Crenulatum*	6 (beneath outback plateau)	78°54.434″N 12°00.156″E
Hinter-15a	*Klebsormidium* cf. *dissectum*	6 (beneath outback plateau)	78°54.434″N 12°00.156″E
A1-1a	*Klebsormidium* cf. *flaccidum*	7 (Ardley)	62°12.765″S 58°55.888″W
A1-2a	*Klebsormidium* cf. *Montanum*	7 (Ardley)	62°12.765″S 58°55.888″W
A4II2b	*Klebsormidium* cf. *flaccidum*	7 (Ardley)	62°12.765″S 58°55.888″W
APII2b	*Klebsormidium* cf. *flaccidum*	8 (Ardley Palaeo)	62°12.775″S 58°56.816″W
AB01-1all	*Klebsormidium* cf. *flaccidum*	9 (King George)	62°13.315″S 58°57.289″W
AB01-2b	*Klebsormidium* cf. *montanum*	9 (King George)	62°13.315″S 58°57.289″W
AB01-13a	*Klebsormidium* cf. *klebsii*	9 (King George)	62°13.315″S 58°57.289″W
AB01d	*Klebsormidium* cf. *dissectum*	9 (King George)	62°13.315″S 58°57.289″W
AB01m	*Klebsormidium* cf. *montanum*	9 (King George)	62°13.315″S 58°57.289″W

Station numbers 1–6, Arctic study site; station numbers 7–9, Antarctic study site. For comparison see also the map of ***Figure 1****.*

3N-BBM-agar (Starr and Zeikus, 1993; BBM modified by the addition of triple nitrate concentration). After 10 days a small amount of pre-culture biomass was transferred in a new 24-well plate with four replicates for each strain. Chlorophyll *a* fluorescence was measured (excitation 440 nm, emission 680 nm, top read function) each day with a SpectraMax M2e multiplate reader (MPR; Molecular Devices, Biberach, Germany) using the software SoftMax Pro version 5.4 (Molecular Devices, LCC, San Jose, CA, United States). Dark-incubation of 10 min was performed in order to open all reaction centers of photosystem II before starting each measurement. Increasing fluorescence was detected as relative fluorescence units (RFUs) and the fluorescence measured directly after the inoculation serving as a starting value. Each strain was tested four times. Calculation of growth rate for each of the four replicates was performed according to the well-established protocol by Gustavs et al. (2009). Fluorescence Ft at a given time point t are calculated as $F_t = F_0 e^{\mu t}$ with F_0 as initial fluorescence and μ (d^{-1}) as growth rate in the respective time interval. For the calculation of μ the measured chlorophyll *a* fluorescence values were fitted with the equation described above. The fitting was based on the sum of the mean square error A_{Fl} which was calculated as $A_{Fl} = (F_t - F_{t,cal})^2$ with F_t (RFU) as fluorescence at a given point in time t and $F_{t,cal}$ (RFU) as calculated fluorescence at a given point in time t with five subsequent fluorescence values. A_{Fl} was minimized by the Microsoft Excel add-in Solver with the model "GRG- non-linear." Optimum growth rate was defined as growth rate values >80% according to Gustavs et al. (2010).

Multivariate Statistics

Multivariate analysis was conducted using the statistical programs PRIMER 6. Non-metric multi-dimensional scaling (MDS, Kruskal and Wish, 1978) was based on square root transformed data and Bray-Curtis similarity and the significance of similarity was tested by using ANOSIM permutation test (Clarke and Green, 1988; Clarke, 1993).

RESULTS

The results showed a wide range in the capability to growth with increasing temperatures in all investigated *Klebsormidium* strains (6–28°C, **Figure 2**). All strains grew at minimum temperature of 6°C but only four strains were able to grow at the maximum temperature of 28°C (AD-16a *K. nitens*, EiE-15a *K. dissectum*, AB01-2b *K. montanum*, AB01-13a *K. klebsii*). Two growth patterns along the temperature gradient were observed and can be characterized as a plateau-like growth optimum and a more pronounced, sharp optimum. AB01-2b *K. montanum*, EiE-14a *K. subtile*, Hinter-11aII *K. crenulatum*, Hinter-15a *K. dissectum*, EiE-15a *K. dissectum*, and AD-16a *K. nitens* exhibited a plateau-like growth optimum, whereas EiE-1B *K. subtile*, Björn-1C *K. subtile* and AB01-1D *K. dissectum* showed a more pronounced, sharp optimum. Conspicuous differences in optimum growth temperature of *K. montanum* and *K. dissectum* strains indicated an infraspecific physiological plasticity. Growth rates at optimal temperatures of *K. montanum* varied between 18 and 26°C. Both strains were isolated from Antarctic BSCs. Similar individual growth pattern was observed in *K. dissectum* strains, which exhibit optimum growth temperatures between 18 and 23°C. All investigated *K. dissectum* strains were found in Arctic biocrust communities. An overview of the optimum growth temperatures of all strains is presented in **Figure 3**. The MDS analysis and ANOSIM test (global R value = 0.01, p = 0.424) show neither significant grouping by Polar Regions nor by species (**Figure 4**).

DISCUSSION

The investigated Polar *Klebsormidium* strains exhibited relatively high optimum growth temperatures between 18 and 26°C and

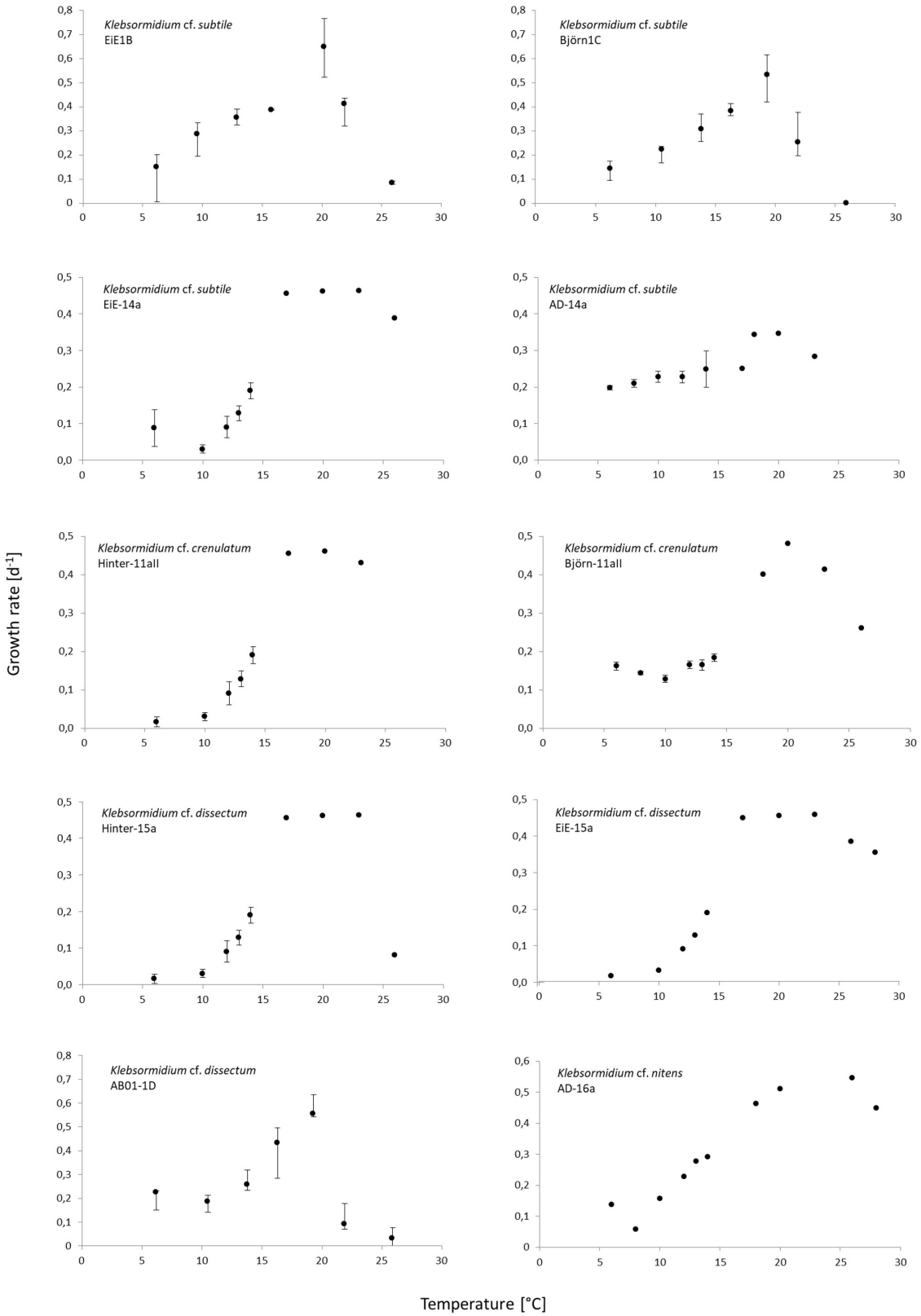

FIGURE 2 | Continued

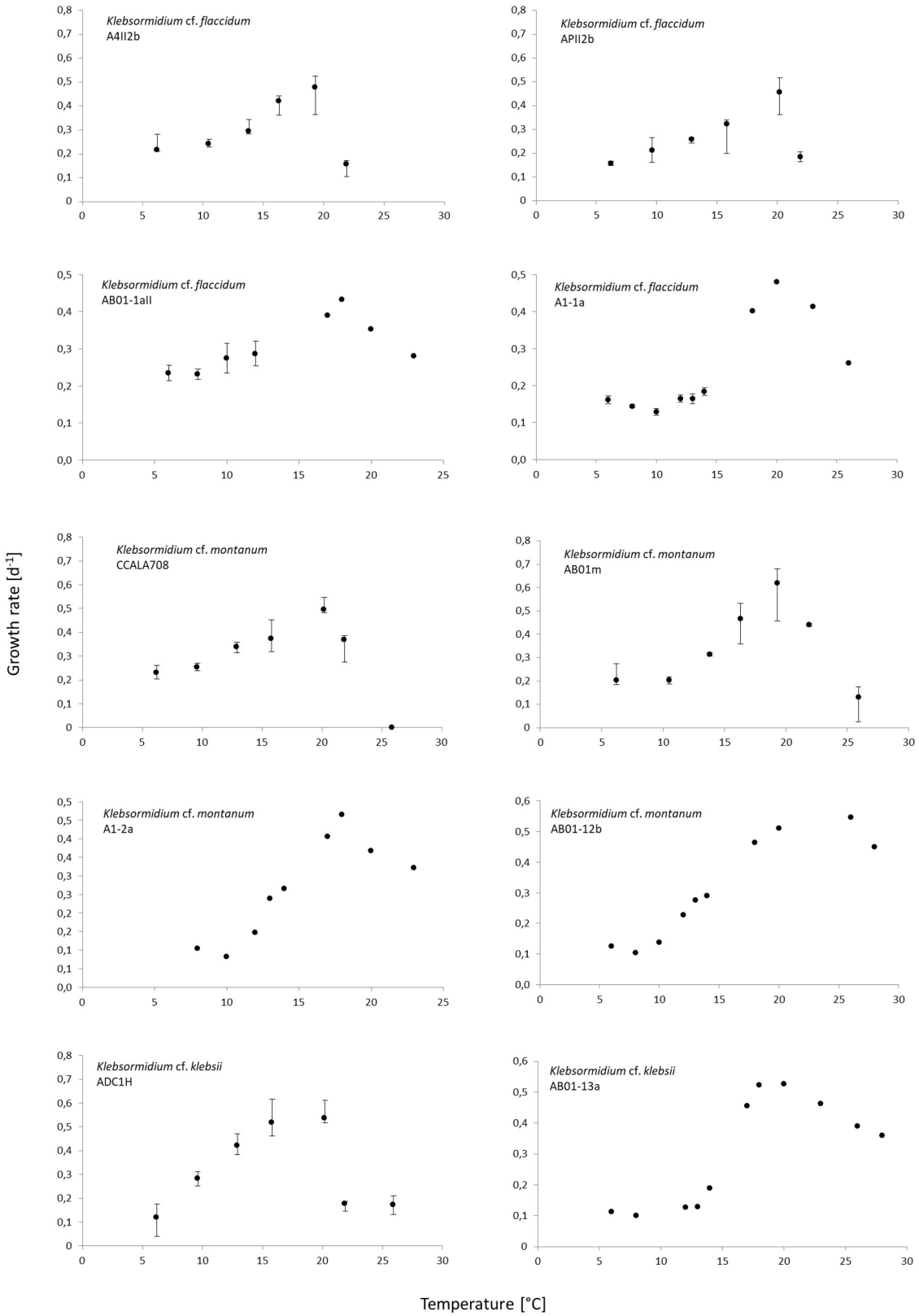

FIGURE 2 | Median growth rate μ [d^{-1}] as response against temperature of all 20 *Klebsormidium* strains. Error bars mark maximum and minimum growth rates of the four replicates for the respective temperature. The temperature range of a few strains were a little different, please keep in mind the *y*-axis.

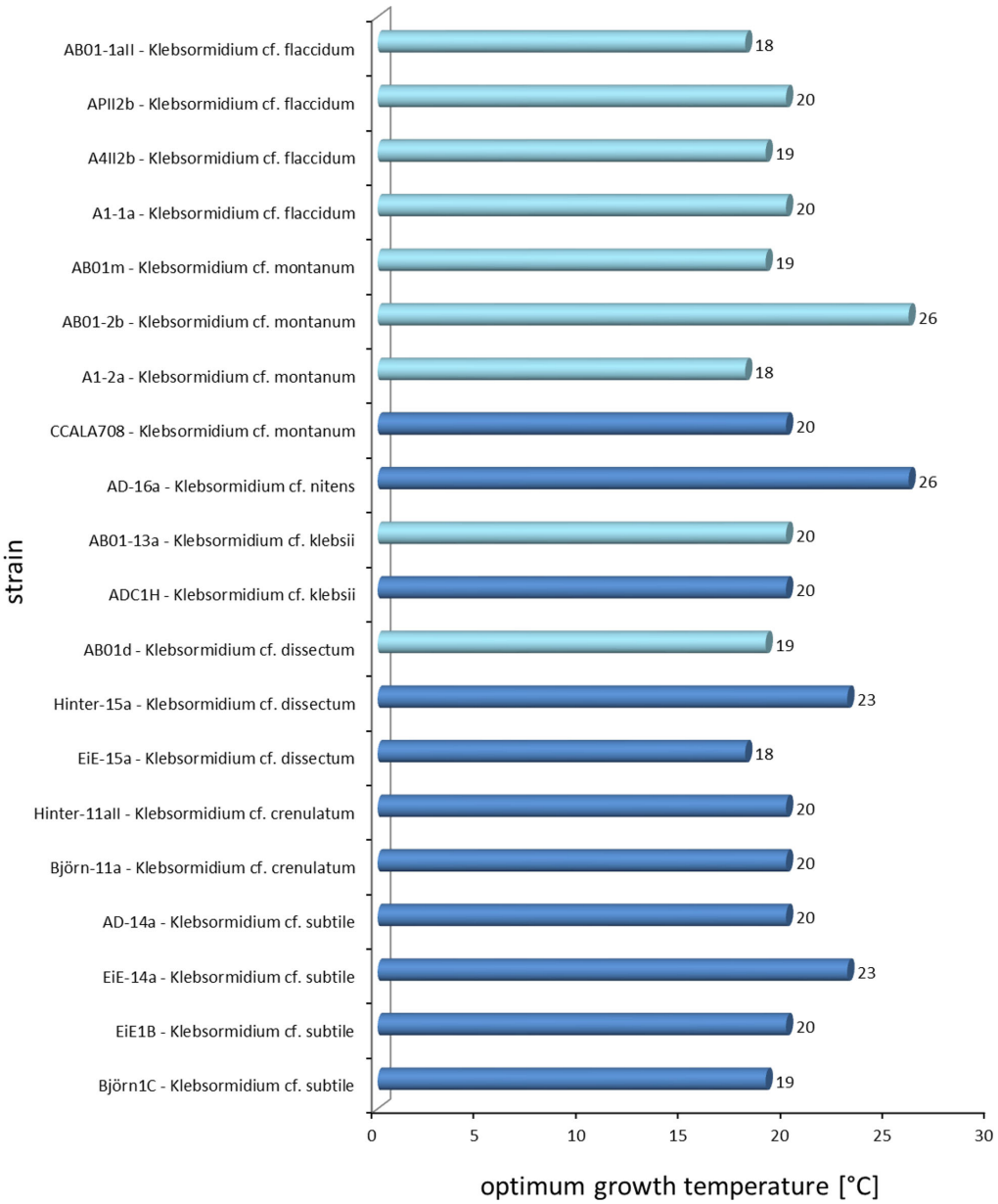

FIGURE 3 | Overview of the optimum growth temperatures of all 20 *Klebsormidium* strains. Light blue = Antarctic strains, dark blue = Arctic strains.

hence, were characterized as rather psychrotolerant taxon than as a psychrophilic or even cryophilic alga. Psychrotolerant organisms grow optimally at 20 to 25°C but are able to survive at temperatures below 0°C (Morita, 1975). Psychrophiles *sensu stricto* have a growth optimum temperature at <15°C and cryophilic organisms prefer temperatures below −10°C (Morita, 1975; Elster, 1999). Both Arctic and Antarctic Klebsormidium strains showed wide ranges of temperatures. Ubiquitous distribution of Klebsormidium is known and it can be concluded that members seem to be ecophysiological generalists with eurythermal growth response pattern. Most of the strains exhibit optimum temperature of 20°C, which coincides with former literature values from the Alps (20°C, Karsten et al., 2010; Karsten and Holzinger, 2012). Furthermore, results presented in this study were comparable with findings on Klebsormidium strains from other biogeographical regions, such as America (median 19°C, Donner et al., 2017) and Germany (20°C, Karsten and Rindi, 2010), and strengthen the hypothesis. Further data on the temperature requirements for growth in both closely related genera of the Klebsormidiophyceae Interfilum, Entransia as well as Hormidiella and other green algae are rare or still missing.

The Arctic Svalbard has a more fluctuating climate with higher summer and lower winter temperatures compared to the Antarctic South Shetland Islands. Furthermore, noticeable

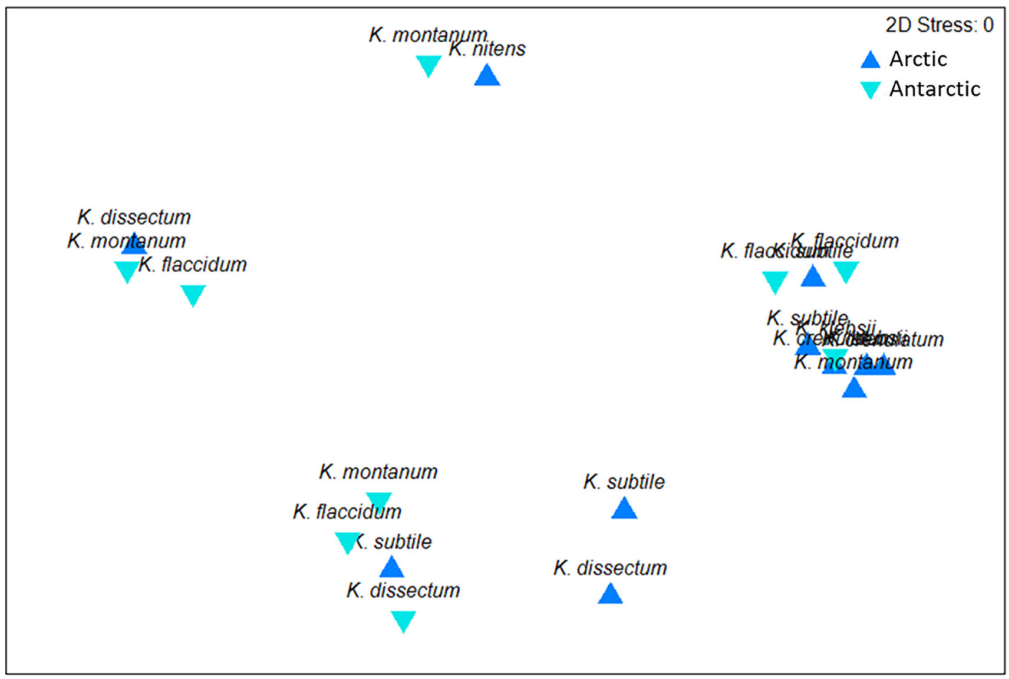

FIGURE 4 | MDS plot based on square root transformed data and Bray-Curtis similarity. Comparison of Arctic and Antarctic *Klebsormidium* strains by their optimum growth temperatures. The stress value represents the quality of the graph (0 = perfect, 0.05 = good, 0.2 = poor).

environmental differences between the Arctic and Antarctica based on their cold water and low temperature history were reported (Zacher et al., 2009). Antarctica has a much longer cold water history of about 23 Mio. years, while the geological cold water history of the Arctic is still younger (ca. 2 Mio years, Sabbe et al., 2003). However, this environmental differences could not be linked to the findings presented in this study because there are no significant differences between Antarctic and Arctic strains. In addition, BSCs microalgae such as *Klebsormidium* have to cope with microclimatic factors, which might explain the local as well as infraspecific differences of optimum growth temperatures.

The differences between the optimum growth temperatures and the air temperatures in the Polar Regions (average 7°C in Svalbard) initially seem to be large. However, investigations on vegetation mats in Svalbard, which described potentially BSCs, revealed an increase by 35% of the temperatures within the vegetation mats (Coulson et al., 1993). These findings constitute a rise by about 5°C during summertime and caused by heat emission due to biological activity (Coulson et al., 1993). These higher temperatures within BSCs in general might explain the ascertained optimum growth temperatures of *Klebsormidium* strains.

A temperature increase up to 7.7°C is predicted for the Arctic region, which will lead to a decline of snow cover and glacier retreat. Our findings indicated that Polar *Klebsormidium* strains are able to survive such changing conditions. Consequently, it allows to presume that Polar *Klebsormidium* strains might benefit from higher environmental temperatures and colonies will increase. This is already indicated by the conspicuously high abundance of *Klebsormidium* species in Arctic as well as Antarctic BSCs (Elster et al., 1999; Kaštovská et al., 2005; Borchhardt et al., 2017a,b, 2018).

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

AUTHOR CONTRIBUTIONS

SG-P performed the experiments and analyzed the data. NB collected the samples, cultivated and isolated *Klebsormidium* strains, identified species, performed experiments, analyzed data, created figures and table, and wrote the first draft of the manuscript. Both authors edited and revised the manuscript and approved the publication.

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frontiers in Ecology and Evolution

ORIGINAL RESEARCH
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Modest Residual Effects of Short-Term Warming, Altered Hydration, and Biocrust Successional State on Dryland Soil Heterotrophic Carbon and Nitrogen Cycling

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Biological soil crusts (biocrusts) on the Colorado Plateau may fuel carbon (C) and nitrogen (N) cycling of soil heterotrophic organisms throughout the region. Late successional moss and lichen biocrusts, in particular, can increase soil C and N availability, but some data suggest these biocrust types will be replaced by early successional cyanobacterial biocrusts as the region undergoes warming and aridification. In this study, we evaluated the short-term interactive effects of biocrust successional state and elevated temperature on soil heterotrophic C and N cycling (specifically, soil respiration, N_2O emissions, microbial biomass C and N, and soluble C and N). We collected soils following an 87-day greenhouse mesocosm experiment where the soils had been topped with different biocrust successional states (moss-dominated, cyanobacteria-dominated, or no biocrust) and had experienced different temperatures (ambient and warmed), under an artificial precipitation regime. Following this pre-incubation mesocosm phase, the soils were assessed using a short-term (2-day) laboratory incubation to determine the cumulative effect of the elevated temperature and altered biocrust successional state on the temperature sensitivity of soil heterotrophic C and N cycling. We found that there were interactive effects of biocrust successional state and exposure to warmer temperatures during the mesocosm phase under greenhouse conditions on the rate and temperature sensitivity of soil heterotrophic C and N cycling in laboratory incubations. Soils collected from beneath late successional biocrusts exhibited higher C and N cycling rates than those from beneath early successional crusts, while warming reduced both the magnitude and the temperature sensitivity of C and N cycling. The inhibiting effect of warming, was most evident in soils from beneath late successional biocrusts, which, during the mesocosm phase, also exhibited the greatest reductions in gross primary production and respiration in response to the warming treatment. Taken together, these data suggest that an overall effect of

climate warming may be increasing resource limitation of the soil heterotrophic C and N cycles in the region, which may magnify alterations associated with the changes in biocrust community structure documented in previous studies. Overall, results from this study suggest that soil heterotrophic biogeochemical cycling is affected by interactions between temperature and the biocrust community that lives atop the mineral soil, with important implications for C and N cycling into the future.

Keywords: biological soil crust, climate change, soil respiration, N_2O emission, microbial biomass, carbon, nitrogen, temperature sensitivity

INTRODUCTION

Biological soil crusts (biocrusts) are fundamental members of dryland ecosystems (Weber et al., 2016b), and affect ecosystem functions of dryland soils in ways that vary with biocrust community composition and exposure to altered climate (e.g., Belnap et al., 2016; Reed et al., 2016). Biocrust community composition within a particular ecosystem tends to develop in predictable sequences (Weber et al., 2016a), such that ecological succession can be a useful, if somewhat simplified approach for evaluating and forecasting changes in biocrust function in response to perturbation (Housman et al., 2006; Langhans et al., 2010; Lan et al., 2013; Read et al., 2016). Recent evidence suggests climate changes in many deserts may result in a systemic shift from a 'later' successional-state community of mosses and lichens toward an 'earlier' successional-state community, primarily composed of cyanobacteria (Escolar et al., 2012; Ferrenberg et al., 2015; Maestre et al., 2015; Reed et al., 2016), similar to changes induced by physical disturbance (e.g., vehicle and livestock trampling; Weber et al., 2016a). These effects of climate change and land-use intensification on biocrust communities may extend to the global scale (Rodriguez-Caballero et al., 2018). Because biocrust succession – which in many drylands is a progression from bare soil to cyanobacterial biocrust to lichen, and/or moss biocrust – causes soil stability to increase dramatically (Pietrasiak et al., 2013; Gao et al., 2020), along with soil carbon (C) and nitrogen (N) inputs and pools (e.g., Housman et al., 2006; Guo et al., 2008; Zhou et al., 2020), alterations of biocrust successional state in response to a warmer climate may have significant effects on belowground soil processes.

The Colorado Plateau maintains notable biocrust communities reported to play myriad critical roles in ecosystem function (Belnap et al., 2016). Biocrusts are particularly important at lower to mid-elevations (i.e., 900–2,100 m) within the Colorado Plateau Desert, where biocrust-dominated interspaces between vascular plants can represent a large to dominant category of land cover (>60% e.g., Torres-Cruz et al., 2018). Biocrust functions include regulating fertility of soils beneath crusts (e.g., Ferrenberg et al., 2018) and climate-induced losses of moss and lichen biocrusts have been linked to shifts in soil C and N pools (Reed et al., 2012; Maestre et al., 2013). The heterotrophic microbial communities that live below biocrust also play fundamental roles in ecosystem function (e.g., Castillo-Monroy et al., 2011), however, our understanding of the relationship between aboveground biocrust physiology and belowground heterotrophic biogeochemistry is notably poor. An improved understanding of these linkages is critical considering that altered biocrust function as a result of warming may induce a cascade of changes related to soil fertility (Beraldi-Campesi et al., 2009), plant community structure (Havrilla et al., 2019), C storage and efflux (Darrouzet-Nardi et al., 2015, 2018), hydrology (Eldridge et al., 2020), and soil stability (Gao et al., 2020) - all of which could have substantial negative effects on dryland function. This region has warmed ∼1°C over the previous 50 years, and is projected to warm between 2 and 5°C over the remainder of the 21st century (IPCC, 2013). While precipitation forecasts are much more variable, this warming is predicted to drive an increase in soil dryness (Schlaepfer et al., 2017). Thus, predicting soil biogeochemistry in response to altered climate in these landscapes will require an improved understanding of soil biogeochemical pools and fluxes associated with biocrust and climate-induced changes to their community composition.

A substantial portion of the organisms found in biocrust communities are photoautotrophic and, similar to vascular plants, these photoautotrophs provide organic C to heterotrophs in soils via exudation and turnover. Photosynthetic rates per unit area of biocrust vary widely in relation to the dominant autotroph present, with biocrust mosses exhibiting photosynthetic rates comparable to vascular plants (e.g., max = 3.8 μmol CO_2 m^{-2} s^{-1}), while uptake of CO_2 in biocrusts dominated by lightly-pigmented cyanobacteria can be barely distinguishable from bare soil fluxes (e.g., max = 1.1 μmol CO_2 m^{-2} s^{-1}) (Tucker et al., 2019). Some fraction of the CO_2 fixed by biocrust organisms may be incorporated in the soil organic matter (SOM) pool via exudation and leaching (Swenson et al., 2018), litter inputs, and herbivory by soil invertebrates (Vaculik et al., 2004) among other possible pathways. Given these input pathways, C originating in biocrusts may provide the main growth and respiration substrate for heterotrophic soil microbes living in and beneath the biocrust. In these cases, changes to C inputs, either via shifts in biocrust community structure or via changes in physiology of dominant biocrust autotrophs, may result in alternate patterns and temperature sensitivity of soil heterotrophic C cycling.

The role of biocrusts in soil N cycling may be somewhat more complex than in C cycling (Barger et al., 2016). Globally, semi-arid ecosystems, including the Colorado Plateau in the southwestern United States, tend to have relatively low concentrations of soil N (Post et al., 1985). Biocrusts provide a primary input of N to these ecosystems (e.g., Evans and Ehleringer, 1993), in large part because N_2-fixing biocrust organisms release substantial amounts of both inorganic and

organic N into the soil (e.g., Johnson et al., 2007), especially in response to wetting after a dry period (see Barger et al., 2016 for a review of biocrust N release into soil). Some biocrust organisms are highly effective N_2-fixers (e.g., *Collema* spp. lichens, and later successional biocrust in general), while others show much lower rates and are likely acquiring most of their N from the soil environment (Hawkes, 2003; Yeager et al., 2004; Housman et al., 2006; Torres-Cruz et al., 2018). Biocrusts may also lead to accelerated loss of soil N via emission of N_2O, NO_2, and HONO (Barger et al., 2005; Strauss et al., 2012; Abed et al., 2013; Weber et al., 2015). Furthermore, soil heterotrophic C and N cycling are likely to vary significantly as a function of this input of N (Schaeffer and Evans, 2005). While the importance of biocrust for N cycling in the uppermost soil layers (<5 mm depth below the biocrust) has been documented, the contribution of biocrusts to N cycling in the bulk soil is less well understood (Johnson et al., 2007; Castillo-Monroy et al., 2010; Delgado-Baquerizo et al., 2013; Barger et al., 2016; Zhou et al., 2020), as is our understanding of how biocrust type and physiology affect soil heterotrophic N cycling.

Here, we aimed to improve our understanding of the linkage between biocrusts and soil C and N cycling driven by heterotrophic microbes, especially with regard to the temperature sensitivity of these pools and their fluxes. In particular, we assessed how respiration and N_2O emissions from soil heterotrophic organisms, alongside changes of pools of C and N within the soil, were related to the biocrust organisms at the soil surface, and to the temperature regime these organisms experience. Our goal was to elucidate a set of mechanisms underlying the effects of climate warming (and resultant altered hydration) and associated changes in biocrust community on soil C and N cycling. We evaluated heterotrophic C and N cycling for soils collected from a mesocosm experiment focused on ambient and warmed (+5°C) soils of three cover types: bare soil, lightly-pigmented cyanobacterial 'early successional' biocrusts (e.g., *Microcoleus* spp.), and moss-dominated 'late successional' biocrusts (described in Tucker et al., 2019). Soils collected from beneath biocrusts that experienced distinct temperature treatments were subjected to a series of short-term laboratory incubations to assess the rates and temperature sensitivity of soil respiration, N_2O emission, depletion of soluble organic C and N, and changes in microbial biomass C and N. We hypothesized that late successional (i.e., moss-dominated) biocrust would support higher rates of soil heterotrophic C and N cycling compared to early successional (i.e., cyanobacterial) biocrust, which, in turn, would be higher than bare soil. In contrast, we hypothesized that, overall, the warming treatment would slow soil heterotrophic C and N cycling, and this reduction would be greatest under late successional biocrust, which have been shown to be more negatively affected by warming in the field (Ferrenberg et al., 2015). We further hypothesized that, as soil heterotrophic organisms are exposed to warmer temperatures, they would become less sensitive to increased temperature (apparent acclimation or adaptation), which is a notable feature of soil microbial respiration across drylands globally (Dacal et al., 2019), and that these effects would be at least partially driven by a reduction of C substrate availability. It is important to note that during the mesocosm phase of study, the warming treatment resulted in a suite of changes, including reduction of biocrust activity and a 13–32% reduction of the duration of hydration (Tucker et al., 2019), which could have equal or greater impact on the soil heterotrophic community than the direct impact of warming. Thus, in this paper we are evaluating responses to this suite of warming associated changes, rather than exclusively the response to warming.

MATERIALS AND METHODS

Greenhouse Mesocosms

The biocrust physiological and modeling data from this mesocosm are described in Tucker et al. (2019), and complete experimental details can be found there. Briefly, samples were collected from a cool desert ecosystem on the Upper Colorado Plateau (36.675 N, -109.416 W; near Castle Valley, UT, United States). MAT for the surrounding area is 13°C and MAP is 269 mm (based on 1981–2010 data; Western Regional Climate Center 2014). Soils are shallow and classified as sandy loam, calcareous, Rizno series Aridisols, and vegetation is dominated by the native perennial grasses *Achnatherum hymenoides* and *Pleuraphis jamesii*, the perennial shrub *Atriplex confertifolia*, and the exotic annual grass *Bromus tectorum*. Biocrust communities at the site are dominated by the cyanobacterium *Microcoleus* spp., the moss *Syntrichia caninervis*, and the cyanolichens *Collema tenax* and *C. coccophorum*. These species are common in drylands worldwide and represent widespread biocrust functional types (Bowker et al., 2016).

For the initial mesocosm phase (**Figure 1**; Tucker et al., 2019), we assembled mesocosms from biocrust and soil collected from multiple locations within a single drainage with biocrust communities clearly dominated by either early or late successional communities (i.e., communities dominated by lightly-pigmented cyanobacteria or by moss, respectively). Early cyanobacterial crusts were distinguished from bare soil based on three factors (1) the soil formed a distinct crust layer, (2) inspection of plates of this crust revealed numerous filaments hanging from the base of the crust, and (3) this crust layer exhibited some cohesion when wetted. Biocrusts representing early or late successional states were carefully separated from sub-crust soil by inserting a thin metal plate <1 cm below the identified bottom of the biocrust. The sub-crust soil was collected to a depth of 5 cm and homogenized across both successional categories. Homogenized soil was added to a depth of 6 cm to a total of 150 plastic pots (8.5 l × 8.5 w × 7 h cm) with holes in the bottom to allow water to freely drain, and with mesh screen to prevent soil loss with drainage. The soil was then covered by either early or late biocrust or was left bare (Bare soil) (n = 50 for each cover type). Mesocosms were then transferred to the research greenhouse at the US Geological Survey office in Moab, UT, United States. Once inside the greenhouse, mesocosms were watered to holding capacity for 2 days and then allowed to dry for 8 days under a common temperature regime.

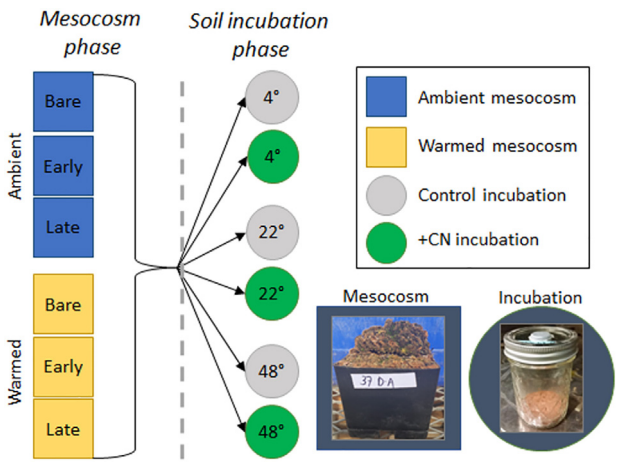

FIGURE 1 | Depiction of the overall experimental design. During the 87-day mesocosm phase, biocrust mesocosms, which represented a homogenous mineral soil covered with no biocrust (Bare), cyanobacteria-dominated biocrusts (Early succession), or moss-dominated biocrusts (Late succession), were maintained in a greenhouse under Ambient or Warmed (~5°C above ambient) conditions. Following this mesocosm phase (for which results are presented in Tucker et al., 2019), a 2-day soil incubation phase was used to assess the temperature and substrate response of soil heterotrophic C and N cycling to the prior mesocosm treatments. In the incubation phase, soil sampled from beneath the biocrust layer for each of five replicate mesocosms from each treatment level was incubated at one of three temperatures (4, 22, and 48°C) either with +CN as glucose and ammonium nitrate or as an unamended control. Soil respiration, N_2O efflux, K_2SO_4-extractable C and N, and microbial biomass C and N concentrations were measured for each sample.

Mesocosm Conditions and Warming Treatment

Following the 8-day acclimation period, 25 mesocosms of each of the 3 cover types were randomly assigned to the Warmed vs. Ambient temperature treatments (**Figure 1** 'mesocosm phase') (Tucker et al., 2019). Warming was implemented using electric and propane heaters (exhausted outside the greenhouse) with thermostatic control was used to maintain a Warmed air temperature ~7°C above Ambient and a Warmed soil temperature ~5°C above the Ambient. Throughout this paper the terms "Ambient" and "Warmed" are used to refer to the mesocosm treatments, not to the temperatures used in the following incubation phase described below (**Figure 1**). During the first week of the experiment (September 16–22, 2015) the Warmed soil temperature range (mean [min, max]) was 29.2 [19.0, 45.3]°C while the Ambient range was 26.0 [16.9, 40.1]°C. By the final week (December 2–8, 2015), the Warmed soil temperature range was 9.0 [1.5, 31.4] °C while the Ambient range was 3.5 [−2.0, 18.7]°C. The maximum soil temperature measured in this study (50.3°C) was lower than the maximum measured at the field site (57.2°C). Mesocosms were watered with 120 ml of DI water once per week throughout the mesocosm phase. A detailed description of the rationale for the watering regime is provided by Tucker et al. (2019).

On December 09, 2015, after 87 days under the experimental treatments in the greenhouse, the mesocosms that had not been destructively sampled during the mesocosm phase were harvested [n = 5 per biocrust (3 levels) and temperature (2 levels) treatment, for a total of 30 harvested mesocosms]. Based on the criteria stated above for distinguishing Bare soil from Early successional biocrusts, none of the Bare mesocosms had formed a biocrust over the course of the incubation, however, we recognize that some cyanobacterial colonization may have occurred. Biocrusts were separated from the sub-crust soils, and the sub-crust soils were then sieved to homogenize and then air-dried for 1 week. Dry soil was refrigerated at 4°C until incubations were initiated on January 11, 2016.

Incubations

From each of the harvested mesocosms (n = 30 based on 2 greenhouse temperatures × 3 biocrust types × 5 replicates), 6 soil samples were collected and were incubated at one of 3 incubation temperatures (4, 22, or 48°C) and one of 2 substrate levels (control, +CN; **Figure 1**). Thus, 180 individual samples were incubated as follows. Air-dried soil (30 g each) was added to 236 ml glass jars with rubber septa ports installed in the lids for collection of headspace gas samples. Soils were then returned to the refrigerator for 1 day to allow them to settle. Soil samples were incubated at either 4, 22, or 48°C for 1 h prior to the addition of C and N amendments and water (**Figure 1** 'soil incubation phase'). Amendments were added as glucose + ammonium nitrate (+CN; 5 mg glucose-C and 1.5 mg ammonium nitrate-N/g dry soil in DI water), or unamended control (DI water only). Incubation trials were conducted prior to the start of this experiment to determine appropriate levels of glucose and ammonium nitrate at which soil respiration would reach maximum (i.e., saturating levels of each amendment). Amendments were added in solution, with 6 ml of deionized H_2O added per incubation jar, bringing each air-dried soil sample to ~20% gravimetric water content. Each jar was then sealed, flushed with compressed zero grade breathing air (76.5–80.5% N_2 and 19.5–23.5% O_2), and incubated at the respective incubation temperature for 1 h, at which point two headspace air samples were collected for analysis of CO_2 and N_2O concentrations. The jars were then unsealed to allow free exchange with the atmosphere and returned to their respective incubation temperature for 3 h, when a subsequent round of headspace gas sampling occurred. This process was repeated at 24 and 48 h. Headspace [CO_2] was measured on a Sable Systems CA-10 CO_2 Infrared Gas Analyzer (Sable Systems International, Las Vegas, NV, United States). Headspace [N_2O] was measured on a Shimadzu GC-14a Gas Chromatograph (Shimadzu Corp., Kyoto, Japan).

Immediately prior to the incubation, and again at 48 h, microbial biomass C and N (MBC and MBN), as well as K_2SO_4-extractable dissolved organic C (DOC) and total dissolved N (TN) were measured via the chloroform fumigation-extraction protocol (Vance et al., 1987). In brief, soil samples were divided into three subsamples: one each for measurement of soil water content, dissolved organic C and total dissolved N concentration, and post-fumigation dissolved organic C and total dissolved N concentration. Chloroform fumigation was conducted for 48 h. Samples were extracted in 0.5 M

K_2SO_4 for 1 h and then filtered using Whatman 42 filter paper. Total dissolved organic C and total N of the extracts were measured on a Shimadzu TOC/TN-VCSH analyzer (Shimadzu Corp., Kyoto, Japan). MBC and MBN were determined as the difference between initial and fumigated samples, corrected by a 0.35 extraction efficiency factor (Voroney et al., 2008).

Statistical Analyses

Cumulative respiration and N_2O efflux were calculated as the weighted sum of flux rates × time across all 4 time points. Exponential temperature sensitivity of trace gas fluxes was evaluated by fitting the log-transformed cumulative flux to a linear function of incubation temperature as equation 1 $\log Z = \alpha_Z \times T + \beta_Z$, where Z is either cumulative respiration or cumulative N_2O efflux, using the glm() function in R statistical software. Q_{10} temperature sensitivity was then calculated as equation 2 $Q_{10} = e^{10\alpha_z}$. Linear temperature sensitivity of changes in the microbial biomass and soil pools of C and N was evaluated by first calculating the change in pool size for each sample as equation 3 $\Delta X = X_{final} - X_{initial}$, where X is either MBC, MBN, DOC, or TN, and then evaluating that change as a linear function of temperature via equation 4 $\Delta X = \alpha_X \times T + \beta_X$. Thus, α_x represents the slope of the change in pool X to a change in soil temperature. We do not account for exponential growth or turnover in this analysis; therefore the microbial biomass response should only be interpreted as a change in biomass, and not a growth rate, and thus is not well-suited for calculating growth efficiency. All pool and flux data were analyzed via permutational analysis of variance after testing revealed violations of assumptions for traditional ANOVA: most response variables (log-transformed in the case of cumulative respiration and N_2O efflux) passed Levene's test for equality of variance, but most fluxes violated Shapiro test for normality of residuals. Therefore, permutational ANOVA was implemented using the aovp function in the lmPerm package in R. Temperature sensitivity estimates (Q_{10} from equation 2 and α_x from equation 4) were also compared using a standard ANOVA after verifying that they met assumptions of normality and equal variance. *Post hoc* pairwise comparisons were conducted using pairwise T-test with a Holm adjustment for multiple comparisons using the pairwise t-test function in R. Significant differences are reported at $\alpha = 0.05$, and marginally significant differences are reported at $0.05 < \alpha < 0.1$. All data analyses were conducted using R statistical software version 3.5.3 'Great Truth' (release 3/11/2019).

RESULTS

Soil C and N Pools Before Incubation

At the end of the mesocosm phase and prior to soil incubations, soil microbial biomass C (MBC) was marginally higher (23%; $p = 0.089$) in Late successional biocrust mesocosms at Ambient temperature (**Figure 2A** and **Table 1**) compared with Bare soil mesocosms at Ambient temperatures, no other differences were significant. Microbial biomass N (MBN) was 55.7 and 32.8% higher in Late relative to Early biocrust or Bare soil mesocosms, respectively ($p < 0.001$ for each; **Figure 2B** and **Table 1**), respectively, but not different among mesocosm temperature treatments. Extractable dissolved organic C (DOC) was not different among treatments. Soil extractable total N (TN) was 96 and 70% higher under Late relatively to Early biocrust or Bare soil treatments, respectively ($p = 0.0075$ and 0.0018, respectively), and was enhanced 42.4% by mesocosm Warming ($p = 0.022$), particularly under Late biocrust (**Figure 2D** and **Table 1**) [i.e., TN in Late successional-Warmed treatments was 71% higher compared with in Late successional-Ambient treatments ($p = 0.047$)].

Unamended Control Soil C and N Pools and Cumulative Fluxes Following Incubation

In the unamended controls, respiration was 100 and 140% greater in soils from Late successional mesocosm soils compared with Early successional or Bare soil mesocosms, respectively ($p = 0.033$ and 0.043), but respiration was not affected by either mesocosm or incubation temperature (**Figure 3** and **Table 2**). In the unamended control N_2O efflux increased with increasing incubation temperature ($p = 0.0026$), but was not affected by other factors (**Figure 3** and **Table 2**). Post-incubation MBC was 67% higher in the mesocosm Ambient compared with Warmed soils, and decreased by 65% at the hottest incubation temperature ($p < 0.001$, **Figure 3** and **Table 2**). Post-incubation MBN was 41.8% greater in Late successional mesocosms compared with Bare ($p = 0.0357$), and decreased by 63% at the hottest incubation temperature ($p < 0.001$). Post-incubation extractable DOC was 103% greater in soils from Late-Ambient than Bare-Ambient mesocosms ($p = 0.026$) and decreased with increasing incubation temperature ($p = 0.032$). Post-incubation extractable TN was 45 and 29% higher in soils from Late compared with Early successional or Bare mesocosms [$p = 0.019$ and 0.097, respectively, and increased with increasing incubation temperature ($p < 0.001$)].

+CN Treatment Soil C and N Pools and Cumulative Fluxes Following Incubation

All pools and fluxes of C and N showed significant increases with the addition of glucose and ammonium nitrate. Cumulative soil respiration was overall 850% higher ($p < 0.001$) in the +CN incubations relative to the unamended controls ($p < 0.001$). In the +CN treatment, respiration increased exponentially with increasing incubation temperature ($p < 0.001$), and respiration at 48° was reduced by 53 and 60.3% between Ambient and Warmed treatments under Late and Early successional biocrust ($p = 0.0011$ and 0.00255), respectively. There was no difference between Ambient and Warmed respiration under Bare soils (**Figure 4** and **Table 3**). N_2O efflux was 482% higher in the +CN incubations than in the unamended control ($p < 0.001$). Cumulative N_2O efflux similarly increased with increasing incubation temperature ($p < 0.001$), and N_2O efflux at 48°C was 44% lower in the Warmed compared with Ambient mesocosms ($p < 0.00135$),

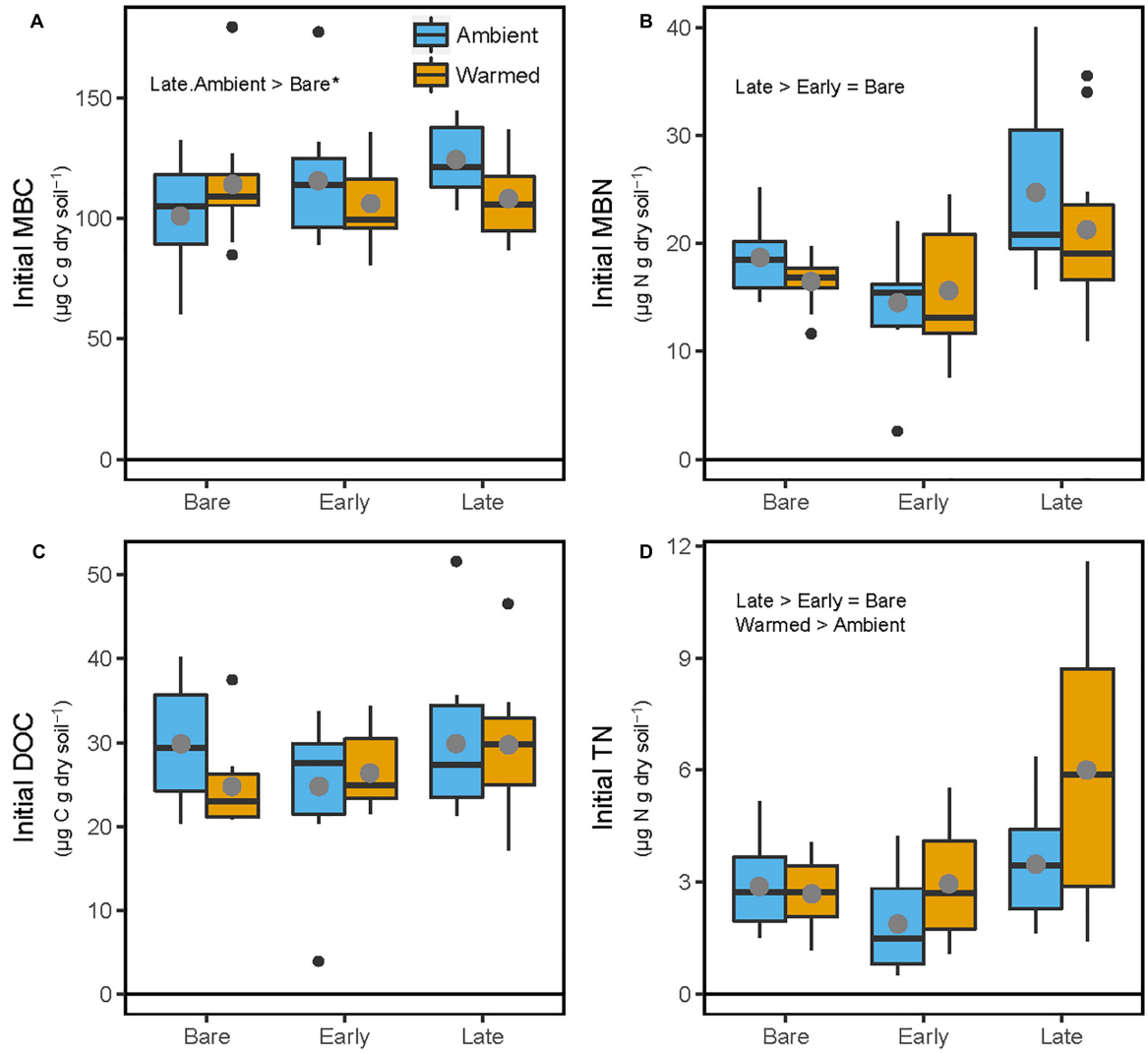

FIGURE 2 | Soil C and N pools following 87-day greenhouse mesocosm incubation, where soils were maintained beneath Bare, Early, or Late successional biocrust states under either 'Ambient' or 'Warm' (+5°C) conditions. **(A)** Microbial biomass C (MBC), **(B)** microbial biomass N (MBN), **(C)** K_2SO_4-extractable dissolved organic C (DOC), **(D)** K_2SO_4-extractable total dissolved N (TN). These values are considered 'Initial' because they represent the conditions prior to the incubation study. Each box represents the median and first (Q1) and third (Q3) quartiles, while bars represent the minimum (Q1 – 1.5 × interquartile range) and maximum (i.e., Q3 + 1.3 × interquartile range), and solid gray circles represent the treatment mean (n = 5). Black dots represent outlier points. Significant differences between prior mesocosm temperature or biocrust successional state treatments are presented using '>' or '<' within each panel. Statistics are presented within the text and in **Table 1**. An asterisk after the comparison signifies a marginal difference ($0.1 > \alpha > 0.05$).

TABLE 1 | ANOVA table for soil C and N pools immediately following the mesocosm phase, prior to the incubation phase.

		MBC_{init}			MBN_{init}			DOC_{init}			TN_{init}	
Factor		**Pseudo-*F***	***P***	±	**Pseudo-*F***	***P***	±	**Pseudo-*F***	***P***	± *	**Pseudo-*F***	***P***
Biocrust		0.831	0.4415	+	**9.19**	**<0.001**		1.681	0.196	+	**8.674**	**<0.001**
$T_{mesocosm}$		0.555	0.4597		0.974	0.328		0.406	0.527	+	**5.275**	**0.0256**
$T_{mesocosm}$ × Biocrust	a	2.52	*0.089*		0.764	0.471		1.077	0.348	b	2.516	*0.0903*

*± indicates the direction of the treatment (factor) effect on the soil pool. Mesocosm treatments assessed include the type of biocrust (Bare, Early, or Late successional) and the temperature of the greenhouse ($T_{mesocosm}$: Ambient or Warmed). Pools include microbial biomass C (MBC), microbial biomass N (MBN), K_2SO_4-extractable dissolved organic C (DOC), and K_2SO_4-extractable total dissolved N (TN). Values that are significant at the α = 0.05 level are in bold, values that are marginally significant ($0.1 > \alpha > 0.05$) are italicized. *Interactions: (a) MBC_{init} is marginally higher in Late-Ambient than other treatments, (b) TN is marginally higher under Warmed than Ambient conditions in Late biocrust.*

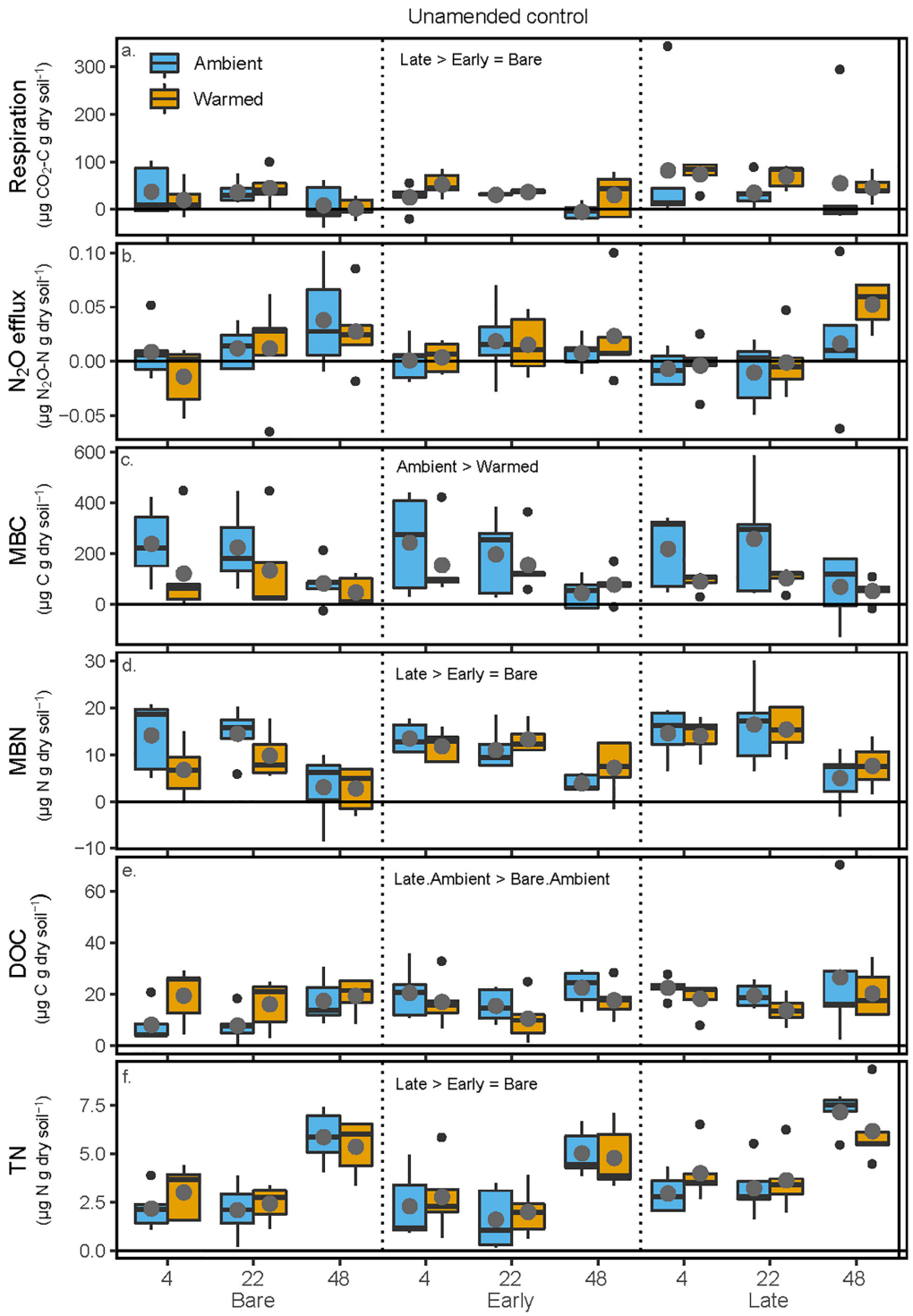

FIGURE 3 | Carbon and N pools and fluxes in soils to which no +CN amendment was added during the soil incubation phase. Shown are **(a)** soil respiration, **(b)** soil N_2O efflux, **(c)** microbial biomass C (MBC) concentrations, **(d)** microbial biomass N (MBN) concentrations, **(e)** K_2SO_4-extractable dissolved organic C (DOC), and **(f)** K_2SO_4-extractable total dissolved N (TN). The *x*-axis is arranged with incubation temperature (4, 22, and 48°C) grouped within the mesocosm biocrust successional treatments (Bare, Early, and Late). Blue boxes show fluxes and pools for the soils from Ambient temperature during the mesocosm phase, while orange boxes are mesocosm Warmed soils. Each box represents the median and first (Q1) and third (Q3) quartiles, while bars represent the minimum (Q1 – 1.5 × interquartile range) and maximum (i.e., Q3 + 1.3 × interquartile range). Gray circles represent mean values (n = 5) and black dots represent outlier points. Significant differences between prior mesocosm temperature or biocrust successional state treatments are presented using '>' or '<' within each panel and statistics are presented within the text and in **Table 2**.

TABLE 2 | ANOVA table for soil C and N pools and fluxes following the soil incubations in unamended control soils.

Factor		Respiration			N_2O efflux			MBC			MBN			DOC			TN		
	±	Pseudo *F*	*P*	±	Pseudo *F*	*P*	±	Pseudo *F*	*P*	±	Pseudo *F*	*P*	±*	Pseudo *F*	*P*	±	Pseudo *F*	*P*
Biocrust	+	**3.765**	**0.026**		0.282	0.755		0.08	0.922	+	**3.493**	**0.0357**		2.305	0.107	+	**8.05**	**<0.001**
$T_{mesocosm}$		0.442	0.359		0.247	0.62	–	**6.345**	**0.0136**		0.536	0.467		0.187	0.667		0.43	0.514
$T_{incubation}$		1.798	0.239	+	**6.482**	**0.0026**	–	**7.532**	**<0.001**	–	**22.32**	**<0.001**	–	**3.625**	**0.032**	+	**45.55**	**<0.001**
$T_{mesocosm}$ × Biocrust		0.492	0.555		1.352	0.265		0.522	0.596		2.141	0.125	**a**	**3.847**	**0.026**		0.004	0.996
$T_{incubation}$ × $T_{mesocosm}$		0.16	0.788		0.68	0.509		1.362	0.263		1.702	0.1896		0.353	0.704		1.778	0.176
$T_{incubation}$ × Biocrust		0.354	0.609		1.141	0.344		0.103	0.98		0.257	0.9044		0.116	0.977		0.258	0.904
3-way		0.344	0.826		0.29	0.88		0.088	0.986		0.176	0.95		0.143	0.965		0.135	0.969

*Respiration and N_2O efflux are cumulative. ± indicates the direction of the treatment (factor) effect on the soil rate or pool. Treatments assessed include the type of biocrust used in the mesocosm (Bare, Early, or Late succession), the temperature treatment of the mesocosm ($T_{mesocosm}$: Ambient or Warmed), and the temperature of the soil incubation ($T_{incubation}$: 4, 22, and 48°C). Values are for soil respiration, soil N_2O efflux, microbial biomass C (MBC), microbial biomass N (MBN), K_2SO_4-extractable dissolved organic C (DOC), and K_2SO_4-extractable total dissolved N (TN). Values that are significant at the α = 0.05 level are in bold, values that are marginally significant (0.1 > α > 0.05) are italicized. *Interactions: (a) DOC is greater under Late successional biocrust than Bare soil at Ambient but not Warmed temperatures.*

while no other factor was significant. In the +CN treatment, MBC decreased by 85% at 48°C ($p < 0.001$) compared with the low temperatures, and was not responsive to other factors. No significant effects of any treatments were apparent in MBN, due to the very high uncertainty in estimates of MBN from soils to which inorganic N was added. DOC declined with increasing incubation temperature ($p < 0.001$), and DOC was marginally higher at lower incubation temperatures in soils from the Ambient compared to the Warmed mesocosms ($p = 0.062$). TN was lower at higher incubation temperatures ($p < 0.001$) and was marginally higher in Ambient compared with Warmed mesocosms ($p = 0.098$).

Temperature Sensitivity of Pool Changes and Trace Gas Fluxes

Overall, the temperature sensitivity of CO_2 and N_2O fluxes and changes in the pool sizes of C and N was much greater (in either the positive or negative direction) for soils subjected to the +CN treatment than the no-amendment controls (**Figures 5**, **6**). The temperature sensitivity of soil respiration (Q_{10R}) was 25.5% lower in soils from Warmed than Ambient mesocosms in the +CN treatment for Late successional biocrust ($p = 0.03$), but this difference was not significant for other biocrust types (**Figure 5** and **Table 4**). There was no effect of mesocosm warming on Q_{10R} in the unamended controls (**Figure 5** and **Table 4**). The temperature sensitivity of soil N_2O efflux (Q_{10N}) was not significantly affected by either mesocosm temperature or biocrust successional state (**Figures 5**, **6**). Microbial biomass declined with increasing incubation temperature, thus the temperature sensitivities of microbial biomass (α_{MBC}, α_{MBN}) were negative (**Figures 5**, **6**). The temperature sensitivity of the changes in the MBC pool (α_{MBC}) was reduced (that is, the pool declined less rapidly) in the Warmed mesocosms compared to the Ambient in the unamended control soils ($p = 0.006$) – the same trend was apparent in the +CN treatment, however, was not significant (**Figures 5**, **6** and **Table 4**). The temperature sensitivity of the change in microbial biomass N (α_{MBN}) was reduced by mesocosm warming ($p = 0.0033$) in the unamended controls but not the +CN treatment, but was not responsive to the biocrust successional state (**Figure 5**). In the +CN treatment the DOC pool showed reduced temperature sensitivity (i.e., α_{DOC} was less negative) in response to mesocosm warming ($p = 0.037$), while the soil TN pool showed marginally reduced temperature sensitivity in response to warming, mostly evident in soil collected from under Early successional biocrust (**Figure 5** and **Table 4**).

DISCUSSION

Overall Findings Related to Hypotheses

During the mesocosm phase of the study, warming and associated drying significantly reduced both CO_2 uptake via photosynthesis and loss via respiration, driving an overall negative C balance and resulting in C starvation, especially for Late successional biocrusts (Tucker et al., 2019). Here we used a set of follow-up soil incubations to determine

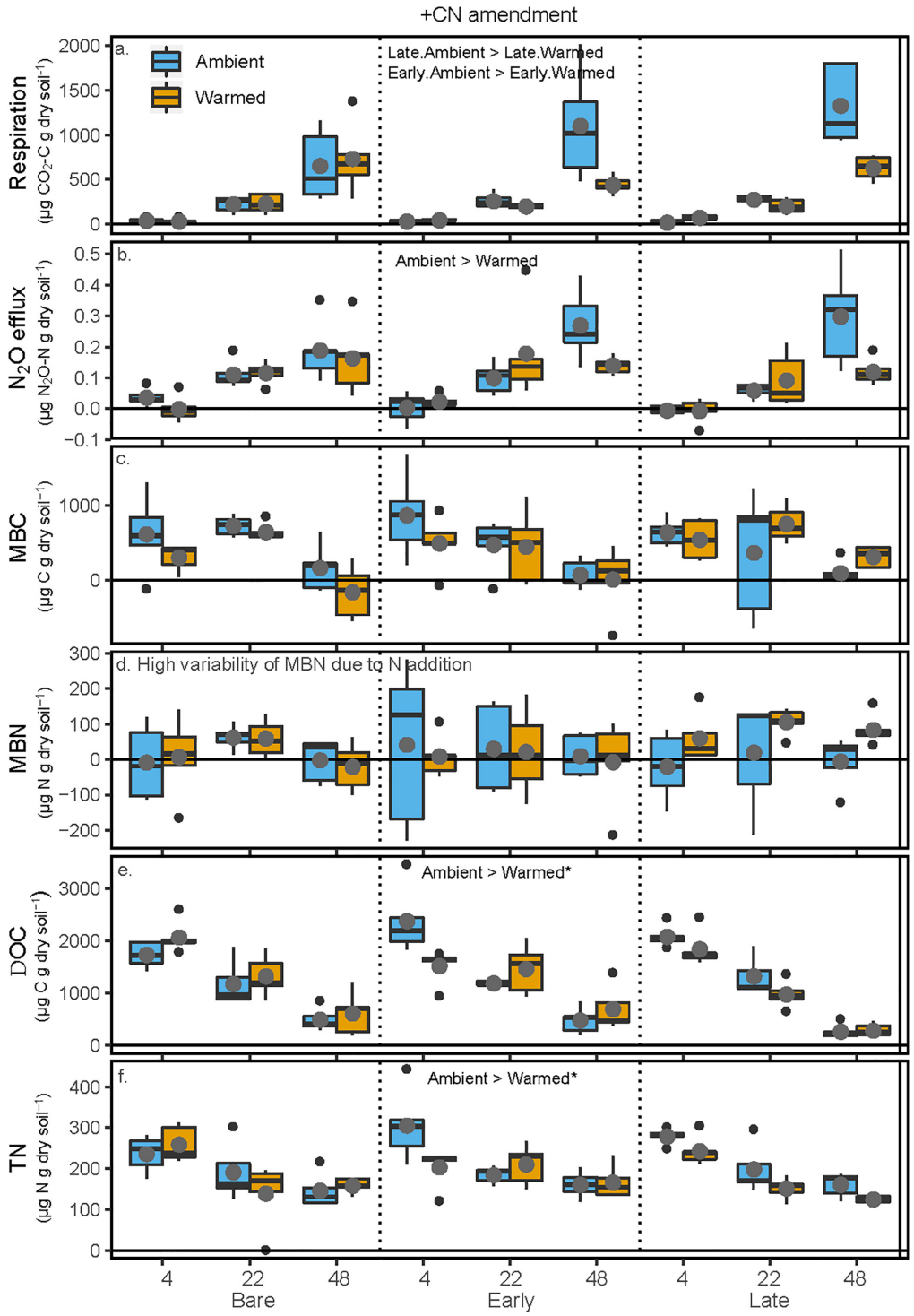

FIGURE 4 | Carbon and N pools and fluxes in soils to which glucose and ammonium nitrate (+CN) were added following the soil incubation phase. Shown are **(a)** soil respiration, **(b)** soil N_2O efflux, **(c)** microbial biomass C (MBC) concentrations, **(d)** microbial biomass N (MBN) concentrations, **(e)** K_2SO_4-extractable dissolved organic C (DOC), and **(f)** K_2SO_4-extractable total dissolved N (TN). The *x*-axis is arranged with incubation temperature (4, 22, and 48°C) grouped by the mesocosm biocrust successional treatments (Bare, Early, and Late). Blue boxes show fluxes and pools for the soils from Ambient temperature during the mesocosm phase, while orange boxes are mesocosm Warmed soils. Each box represents the median and first (Q1) and third (Q3) quartiles, while bars represent the minimum (Q1 – 1.5 × interquartile range) and maximum (i.e., Q3 + 1.3 × interquartile range). Gray circles represent the mean (n = 5) and black dots represent outlier points. Significant differences between prior mesocosm temperature or biocrust successional state are presented using '>' or '<' within each panel. Statistics are presented within the text and in **Table 3**. An asterisk after the comparison signifies a marginal difference ($0.1 > \alpha > 0.05$).

TABLE 3 | ANOVA table for soil C and N pools and fluxes following the soil incubations in soils to which glucose + ammonium nitrate (+CN) was added.

Factor			Respiration			N_2O efflux			MBC			MBN			DOC			TN		
		± *	Pseudo *F*	*P*		± *	Pseudo *F*	*P*	±	Pseudo *F*	*P*	±	*F*	*P*	±	Pseudo *F*	*P*	±	Pseudo *F*	*P*
Biocrust			1.595	0.209			0.865	0.425		0.301	0.741			n.s.		1.715	0.187		1.041	0.3585
$T_{mesocosm}$		–	**9.547**	**0.0028**			2.481	0.119		0.915	0.342			n.s.		0.269	0.605	–	**5.39**	**0.0231**
$T_{incubation}$		+	**92.144**	**<0.001**		+	**43.67**	**<0.001**	–	**17.33**	**<0.001**			n.s.	–	**139.9**	**<0.001**	–	**38.254**	**<0.001**
$T_{mesocosm}$ × Biocrust		**a**	**3.291**	**0.0429**			0.504	0.6063		*2.505*	*0.088*			n.s.		*2.822*	*0.0661*		0.983	0.379
$T_{incubation}$ × $T_{mesocosm}$		**b**	**8.272**	**<0.001**		**b**	**7.186**	**0.0014**		1.69	0.191			n.s.		*2.46*	*0.0926*		0.89	0.415
$T_{incubation}$ × Biocrust			1.261	0.293			0.791	0.535		1.16	0.333			n.s.		0.809	0.534		0.457	0.767
3-way			**2.988**	**0.0243**			1.44	0.2296		0.189	0.9434			n.s.		**3.725**	**0.0082**		**3.243**	**0.0167**

*Respiration and N_2O efflux are cumulative. ± indicates the direction of the treatment (factor) effect on the soil rate or pool. Treatments assessed include the type of biocrust used in the mesocosm (Bare, Early, or Late successional), the temperature treatment of the mesocosm ($T_{mesocosm}$: Ambient or Warmed), and the temperature of the soil incubation ($T_{incubation}$; 4, 22, and 48°C). Values are for soil respiration, soil N_2O efflux, microbial biomass C (MBC), microbial biomass N (MBN), K_2SO_4-extractable dissolved organic C (DOC), and K_2SO_4-extractable total dissolved N (TN). Values that are significant at the α = 0.05 level are in bold, values that are marginally significant (0.1 > α > 0.05) are italicized. *Interactions: (a) warming reduction was greatest in Late successional biocrust, (b) mesocosm warming reduction was greatest at the highest incubation temperature.*

how these effects shape key soil biogeochemical fluxes and pools by applying a range of incubation temperatures and C and N substrates (**Figure 1**). Interestingly, the prior biocrust cover type (Bare, Early successional, and Late successional; as experienced during the Mesocosm phase; **Figure 1**) and the prior temperature regime (Ambient vs. Warmed) remained significantly related to soil respiration and N_2O efflux rates in soil collected from beneath the biocrust even after these past treatments were removed, as did the temperature sensitivity of these fluxes. These effects were modest when compared with the effect of soil incubation temperature, or the addition of C (as glucose) and N (as ammonium nitrate), however, the residual effects of biocrust cover and temperature treatment in the mesocosm did interact with the incubation treatments in ways that help us consider how shifting environments could affect biogeochemical cycles in drylands. Overall, past mesocosm warming reduced both the magnitude and the temperature sensitivity of soil heterotrophic C and N cycling. Late successional biocrust increased C and N pools and fluxes, yet the magnitude of the negative mesocosm warming effect was also greatest beneath Late successional biocrust. Thus, the strongly negative impacts on biocrusts of the warming and watering treatment during the mesocosm phase resulted in a reduced capacity for metabolic activity of a resource-limited soil microbial biomass. These results are consistent with observations of reduced CO_2 cycling by Late biocrusts in response to warming (Darrouzet-Nardi et al., 2015, 2018; Tucker et al., 2019), and reduction of soil and biocrust function in response to warming or altered precipitation observed at the field site from which these soils and biocrusts originated (Reed et al., 2012; Zelikova et al., 2012; Ferrenberg et al., 2015).

Mechanisms Underlying C Cycle Response

The observed responses of the soil heterotrophic C cycle during the soil incubations reflect patterns of CO_2 exchange observed in the greenhouse over the preceding 87-day mesocosm phase (Tucker et al., 2019), when both gross primary production and respiration were inhibited by warming, especially in the Late successional biocrust. Under Ambient conditions, Late successional biocrust mesocosms exhibited a shift to net uptake of CO_2 1 month prior to the end of the mesocosm phase, while Bare soil and Early successional mesocosms, as well as all Warmed mesocosms, continued to experience net CO_2 loss (Tucker et al., 2019). We hypothesized that Late successional biocrust would generate higher rates of heterotrophic soil respiration and larger soil C pools, but that these effects would be diminished under warming. Overall, later successional biocrusts with higher primary productivity would be expected to result in a larger and more active soil heterotrophic community, as was observed. Conversely, the dampened CO_2 exchange that occurred with warming was associated with lower microbial biomass C, a reduced extractable soil C pool, lower respiration rates, and lower temperature sensitivity of respiration. Importantly, these reductions were

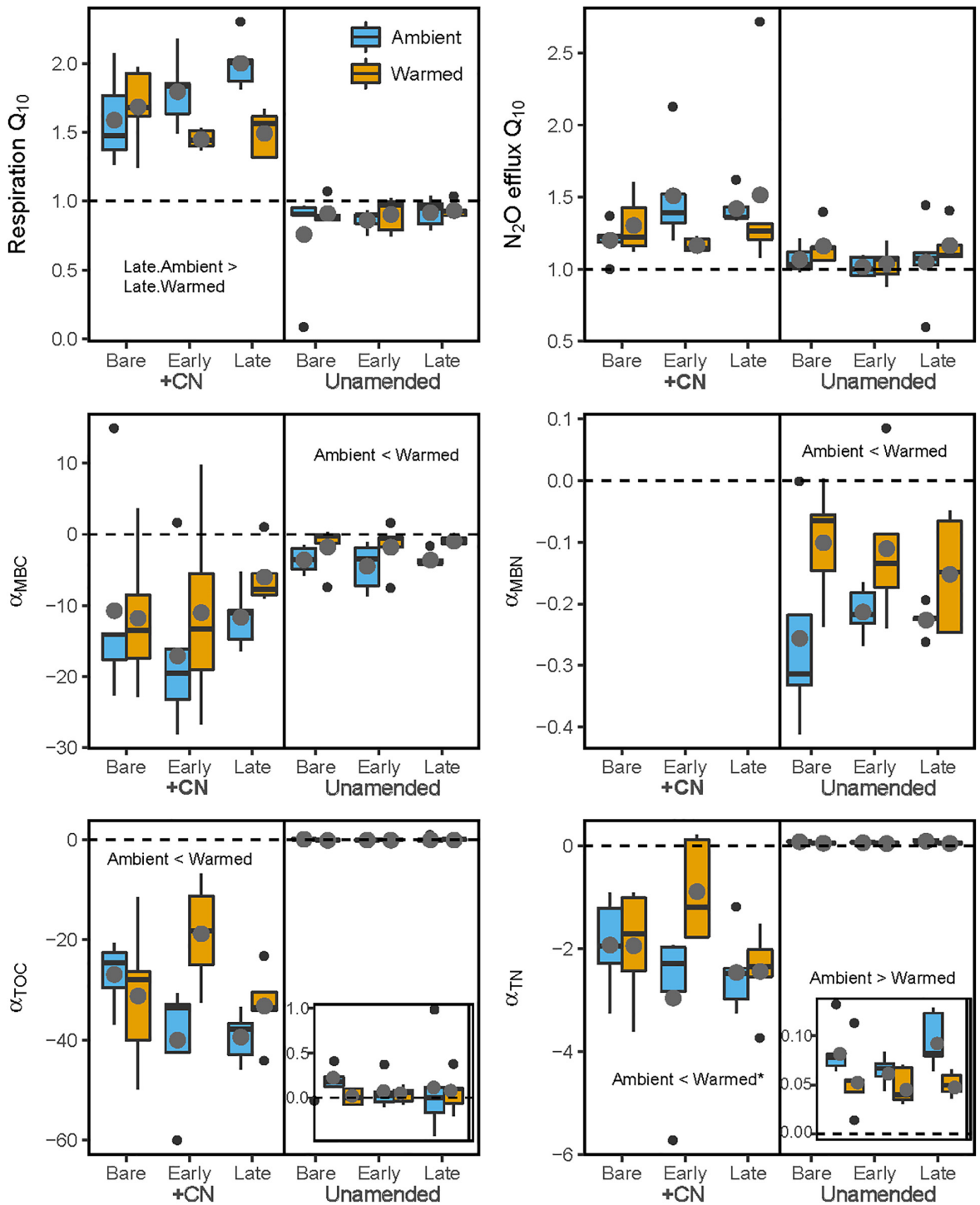

FIGURE 5 | Temperature sensitivity of soil heterotrophic C and N cycling in laboratory incubations. The **(left)** panels represent values for samples with the +CN amendment, while the **(right)** panels shows unamended controls. Blue bars represent Ambient conditions, and orange bars represent the Warmed treatment. Bare, Early, and Late refer to biocrust successional state. Q_{10} refers to the Q_{10} temperature sensitivity of respiration or N_2O efflux, while $\alpha_{(MBC,MBN,\ DOC,orTN)}$ refers to the linear temperature sensitivity of changes in soil microbial biomass C (MBC), microbial biomass N (MBN), K_2SO_4-extractable dissolved organic C (DOC), and K_2SO_4-extractable total dissolved N (TN) pools. Dashed horizontal lines represent the baseline of no temperature sensitivity (either $Q_{10} = 1$ or $\alpha = 0$). Each box represents the median and first (Q1) and third (Q3) quartiles, while bars represent the minimum (Q1 – 1.5 × interquartile range) and maximum (i.e., Q3 + 1.3 × interquartile range). Gray circles represent the mean ($n = 5$), and black dots represent outlier points. Insets in the lower two panels magnify the values for unamended control soils. Significant differences between prior mesocosm temperature or biocrust successional state within each amendment level are presented using '>' or '<' within each panel; these are described in the text and presented in **Table 4**.

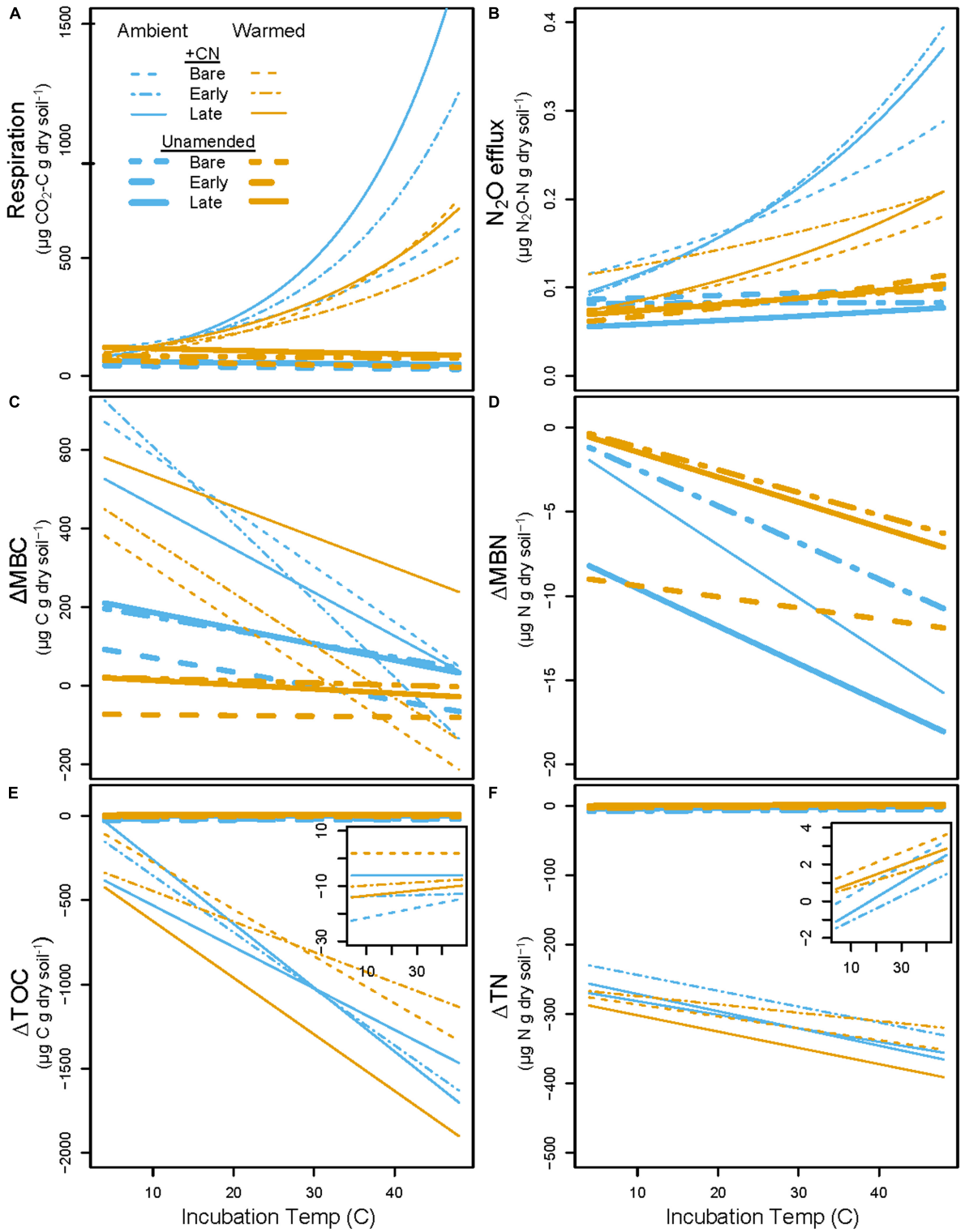

FIGURE 6 | Predicted CO_2 and N_2O cumulative gas fluxes **(A,B)** and changes in pools (Final – Initial; **C–F**) across the range of incubation temperatures based on parameters from exponential and linear regressions. Blue lines represent Ambient treatment values, while orange lines represent Warmed treatment values. Thin lines are +CN soils while thick lines are unamended controls. Dashed lines are Bare, dot-dashed are Early, and solid lines are Late successional biocrust treatments. Inset plots in **(E,F)** show patterns in K_2SO_4-extractable dissolved organic C (ΔDOC) and K_2SO_4-extractable total dissolved N (ΔTN) in the unamended control with a smaller scale. Data used to generate these figures are previously presented as boxplots in **Figures 2–4**, and the differences in slopes between these lines are presented and evaluated in **Figure 5** and **Table 4**. Individual data points and statistics are excluded from this figure to highlight the overall pattern.

TABLE 4 | ANOVA table for temperature sensitivity of soil CO_2 and N_2O fluxes and changes in C and N pools during incubations.

		$T_{mesocosm}$			Biocrust			$T_{mesocosm}$ × Biocrust		
	Temperature sensitivity metric	±	***F***	***P***	±	***F***	***P***	± *	***F***	***P***
Unamended	Q_{10R}		1.18	0.289		0.65	0.53		1.07	0.36
	α_{MBC}	+	**9.27**	**0.006**		0.19	0.83		0.42	0.66
	α_{DOC}		0.93	0.35		0.15	0.86		0.63	0.7
	Q_{10N}		1.65	0.21		0.91	0.42		0.25	0.78
	α_{MBC}	+	**9.27**	**0.006**		0.19	0.83		0.04	0.66
	α_{TN}	+	**11.4**	**0.003**		1.32	0.29		0.85	0.44
+CN	Q_{10R}	−	**8.76**	**0.007**		0.85	0.44	**a**	**4.48**	**0.022**
	α_{MBN}		0.85	0.37		0.60	0.56		0.36	0.70
	α_{DOC}	−	**8.45**	**0.044**		1.7	0.204	**b**	**4.17**	0.028
	Q_{10N}		0.16	0.69		1.06	0.36		1.48	0.25
	α_{MBN}		0.01	0.93		0.64	0.7		0.13	0.88
	α_{TN}	−	*3.12*	*0.09*		0.79	0.47	*c*	*3.08*	*0.065*

*± indicates the direction of the effect. Values that are significant at the α = 0.05 level are in bold, values that are marginally significant (0.1 > α > 0.05) are italicized. $T_{mesocosm}$ refers to the mesocosm temperature treatment (Ambient or Warmed +5°C), Biocrust refers to the biocrust cover type in mesocosms (Bare, Early, and Late successional). Unamended and +CN refer to either no amendment or glucose + ammonium nitrate being added in incubations. $Q_{10(R\ or\ N)}$ refers to the Q_{10} temperature sensitivity of respiration or N_2O efflux, while $\alpha_{(MBC,\ MBN,\ DOC\ ,orTN)}$ refers to the linear temperature sensitivity of changes in soil MBC, MBN, DOC, or TN pools. *Interactions: (a) the decrease in Q_{10R} significant under Late successional biocrust, (b) decrease in DOC temperature sensitivity significant under Early successional biocrust, and (c) decrease in TN temperature sensitivity marginally significant under Early successional biocrust.*

not simply a function of reduced soil C availability and quality; the overall response of C pools and fluxes to incubation temperature was mostly evident under the +CN treatment (**Figure 6**). Thus, in contrast to our hypothesis, the data suggest these patterns were not directly driven by C limitation resulting from inhibited Late successional biocrust productivity. This suggests that subsequent C inputs into the system would have different fates depending on how warming had affected the soil heterotrophic community. These results align with those seen in other ecosystems (Bradford, 2013), and fit within the context of climate change representing a novel effect for biocrusts and the soil beneath them in the dryland biome. Taken together, the data support an emerging understanding of temperature adaptation across dryland ecosystems (Dacal et al., 2019).

In incubations where no amendment C and N was added, CO_2 fluxes were very low and were less sensitive to changes in temperature, indicating that soil heterotrophs were essentially C and/or N-limited, even under Late successional biocrusts, which is consistent with work done at a nearby site (Schaeffer and Evans, 2005). Nevertheless, beneath Late successional biocrusts the soil microbial biomass was larger, more temperature sensitive, and respired at higher rates. We suggest this pattern is linked to Late successional biocrusts providing more substrate throughout the mesocosm phase, given their higher rates of photosynthesis (Tucker et al., 2019). However, this result appears to present a paradox as, during the incubations, soil C cycling appeared very low and minimally responsive to temperature across all biocrust levels in the absence of added C and N. It seems likely that soil microbes rapidly processed the small inputs of C and N derived from biocrust throughout the mesocosm phase, such that, regardless of the biocrust successional state, the soil microbial community persistently depleted C and N inputs. Nonetheless, the microbial community from beneath Late successional biocrusts was able to respond opportunistically to increased resource availability more than were the soil microbes growing beneath Early successional biocrust or Bare soil.

The reduction in temperature sensitivity is consistent with observations of thermal acclimation (e.g., Tucker et al., 2013) or adaptation (e.g., Dacal et al., 2019) in other studies, but we suggest that a different mechanism caused the observed response. Because this pattern was most apparent in the +CN incubations, the data suggest the responses are not a direct function of substrate availability at the moment of measurement, but rather of cumulative substrate availability, which would support a more active or larger opportunistic microbial system to process resources when they do arrive. It is also possible that the physiology of soil respiration changed in response to warming in a way that induced this acclimation-like response (Luo et al., 2001; Tucker et al., 2013), but in this study we did not measure mass-specific respiration, C-use efficiency, or turnover rates in a way that could address this question (e.g., Walker et al., 2018). It is worth noting that microbial biomass decreased in response to increased temperatures in both the mesocosm and incubation phases of this study. Furthermore, microbial biomass in soils from previously warmed mesocosms declined at a reduced rate under higher incubation temperatures compared to biomass in soils from the ambient temperature mesocosms. There are multiple possible explanations of this response, with one of the simplest being that we have observed different phases of an exponential decay curve. Alternatively, the microbial biomass grown under warmed conditions may have a higher temperature optimum for growth and thus experience fewer negative effects under the higher incubation temperatures

(Lipson, 2015). Deciding among these potential mechanisms, or others not considered here, remains a topic in need of further study.

Mechanisms Underlying N Cycle Response

Nitrogen cycling may represent a critical biotic feedback to anthropogenic disturbances in drylands (Schlesinger et al., 1990; Reed et al., 2012). In this study, soil N varied in response to warming and biocrust successional state in ways that may have important implications for dryland responses to future climate change. After the mesocosm phase, soil microbial biomass N concentrations were highest beneath Late successional biocrust but, in contrast with our hypothesis that warming-induced inhibition of Late successional biocrust would reduce microbial N, microbial N concentrations were not reduced by warming. Additionally, total extractable N was highest in Late successional biocrust, and actually increased with mesocosm warming, again contrasting with our hypothesis that soil N would be lost due to warming. Previous work has demonstrated that biocrusts can be a significant source of N to microbes in the soil immediately below the crust (Johnson et al., 2007) and that climate change manipulations can drastically change the quantity and composition of soil N beneath biocrusts (Reed et al., 2012) partly as a function of altered biocrust composition and cover, and presumably changes to new N inputs by biocrusts. During the incubation phase, MBN in the no-amendment treatment was resistant to incubation temperature and not different among mesocosm treatments; the differences among mesocosm treatment levels observed initially were not evident after a 48-h incubation. However, the change in the MBN pool was less sensitive to temperature in the soils that were Warmed during the mesocosm phase compared to the Ambient soils. This result is similar to what was observed with regard to microbial biomass C. Interestingly, soil extractable N increased with warming in the soils to which no amendment had been added, similar to the increase observed in response to mesocosm warming. This was the only soil pool of C or N that increased with warming, and the increase may have been due to the mineralization of the microbial biomass (**Figure 5**).

Gaseous loss of N to the atmosphere is the principal pathway of soil N loss in generally N-limited dryland ecosystems (Peterjohn and Schlesinger, 1990; Schlesinger et al., 1990; Hooper and Johnson, 1999; McCalley and Sparks, 2009; Liu D. et al., 2017). The increased cumulative flux and temperature sensitivity of N_2O emissions we observed when glucose and ammonium nitrate were added to the soil is in line with other studies (e.g., Morley and Baggs, 2010). N_2O emissions from soil occurs via a wide range of biotic pathways, although denitrification is the dominant pathway in most systems (Butterbach-Bahl et al., 2013). N_2O emissions in soils to which no glucose and ammonium nitrate were added were negligible, yet the soil microbial community was able to respond rapidly (<24 h) to these inputs. The amount of N emitted as N_2O (at most 0.5 μg N g dry soil^{-1}) from the +CN treatment was trivial compared to the amount of N added (1.5 mg N g dry soil^{-1}), but when compared to the much smaller pool of extractable N (∼2–4 μg N g dry soil^{-1}) in the initial samples, this flux may represent a substantial potential loss pathway from this ecosystem under the right conditions (Eberwein et al., 2020). This result may partially help to explain the observation that repeated addition of ammonium nitrate over multiple years had minimal impact on the soil microbial community and soil N pools at a nearby site (McHugh et al., 2017).

Conclusion: Consequences for Ecosystem Function in Response to Disturbance and Climate Change

The suite of soil characteristics that changed with warming during an 87-day greenhouse mesocosm experiment induced a reduction of the magnitude and temperature sensitivity of heterotrophic soil C and N cycling, which may signal significant changes to future ecosystem function. Late successional biocrusts, which under ambient temperatures enhanced soil C and N cycling relative to Early successional biocrusts and Bare soil, exhibited the most negative response to warming. Experimental and observational evidence, although from a limited number of sites, suggests that the moss-lichen community composing late successional biocrust is highly vulnerable to warming (Ferrenberg et al., 2015; Maestre et al., 2015; Reed et al., 2016; Mallen-Cooper et al., 2018). Overall then, we might predict a dampened soil C and N cycle in response to warming over time, which would be likely to affect broader ecosystem functions such as plant growth, water retention in soils, as well as influence feedbacks between these soils and the broader climate system. Warming during the 87-day mesocosm phase resulted in reduced magnitude and temperature sensitivity of CO_2 and N_2O emissions from soil during the incubation phase, even in the presence of excess substrate availability. This result likely indicates a reduced capacity for metabolic activity of a resource-limited soil microbial biomass, consistent with the observation of depressed soil C and N cycling in response to warming over time. It is worth noting that soil dissolved N actually increased with warming under Late successional biocrust during the mesocosm phase, likely due to increased mineralization and reduced microbial biomass N uptake, which could in the short term shift N from soils to plants, but could also result in long-term reductions in total soil N storage. While the results described here are from a highly controlled mesocosm and incubation experiment, they nonetheless support a growing understanding, from multiple lines of evidence, that the contribution of Late successional biocrusts to soil ecological functions is reduced by climate warming (Elbert et al., 2012; Reed et al., 2012; Maestre et al., 2013; Liu Y. R. et al., 2017; Ouyang and Hu, 2017; Darrouzet-Nardi et al., 2018; Rodriguez-Caballero et al., 2018; Eldridge and Delgado-Baquerizo, 2019). These results have broader implications for land management across the study region: as ecosystems are increasingly under pressure due to a changing climate, actions that degrade

otherwise intact biocrust communities may accelerate loss of this important component of the soil ecosystem at a time when it is already vulnerable (Weber et al., 2016a), and thus further diminish soil C and N cycling across the region.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

CT, SF, and SR designed the study. CT and SF conducted the experiments. CT conducted data analysis. CT, SF, and SR wrote the manuscript. All authors contributed to the article and approved the submitted version.

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