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Biological soil crust microcolonies reveal how microbial communities assemble following retreat of a High Arctic glacier

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Abstract

Little is known about biological soil crust (BSC) formation during the early stages of primary succession following glacial retreat. Here, we report on focused sampling of twelve discrete BSC colonies near the snout of a retreating glacier in the High Arctic and show that BSC colonies had significantly higher 16S and 18S rRNA gene diversity than the simpler communities of bare sediments sampled next to each colony. Surprisingly, the colonies also had a higher degree of community dispersion than the more clustered bare sediment controls. There were only eight 16S amplicons that showed 100% prevalence in all 12 of the colonies, and the three most abundant of these keystone amplicons were cyanobacteria, including a nitrogen fixing *Nostoc*. The only 18S amplicon common to all colonies was a diatom related to *Sellaphora*. This prominence of phototrophs indicates that early-successional BSC colonies are being supported by photosynthesis rather than ancient- or aeolian-derived organic matter. Co-occurrence network analysis among the phototrophs and fungi identified several potential early-successional soil lichens. Overall, our fine-scaled sampling revealed new insights into community assembly and function in actual communities of interacting microbes (as opposed to mixed communities in bulk soil samples) during the early stages of primary succession.

Keywords: primary succession; carbon sequestration; global warming; cryobiosphere; stochastic community assembly; soil lichens

Introduction

Glacial retreat has formed proglacial forefields where ecosystem succession and pedogenesis can be observed with a chronosequence (space-for-time substitution) approach. Studies applying the chronosequence approach to the earliest stages of succession have revealed that microorganisms can drive carbon and nutrient sequestration that fuels later stages of primary succession of plants (Schmidt et al. 2008, Bradley et al. 2014). Microbial primary succession is particularly evident and important in colder and drier environments—including high latitudes and high elevations, where plant succession can be extremely slow and minimal plant cover may take many decades to develop (Nemergut et al. 2007, Schmidt et al. 2016). In many cold, dry, polar and/or high-elevation environments, biological soil crusts (BSCs) and mosses are the dominant form of ground cover during early primary succession and may even be the climax community in developed soils (Costello et al. 2009, Boy et al. 2016, Solon et al. 2021, Vimercati et al. 2022, Schmidt et al. 2024). BSCs contribute to the process of pedogenesis by adding nutrients to the soil, enhancing water retention, and stabilizing fine-textured soils (characteristic of post-glacial sediments) (Schmidt et al. 2008, Weber et al. 2022, Cao et al. 2023). Despite the importance of BSCs to primary successional processes, there is almost nothing known about how these communities assemble and function on newly uncovered sediments following glacial retreat.

Whilst it is generally accepted that interspecies interactions (such as within lichens and plant associations with nitrogen-fixing bacteria) are important during early successional stages, little is known about how interactions at the microbial-community scale may be driving the early stages of primary succession. A major impediment to understanding microbe-microbe interactions early in chronosequence studies is that the scale of investigation and sample collection is rarely congruent with the scale at which microbes interact with each other in nature. For example, typical chronosequence studies of primary succession are based on analyses of homogenized, bulk samples from the top 5–10 cm of soil that include many different ecological niches, habitat types, and associated microbial communities. If we define a community as "a group of potentially interacting species that co-occur in space and time" (Nemergut et al. 2013), a prin-

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cipal goal of microbial community ecology is to be able to define the boundaries of a community to understand how organisms are interacting with each other to drive processes such as primary succession.

A step towards sampling a community of interacting species is to sample at a fine enough scale to capture potentially interacting species. For example, phototrophic microbes usually drive primary production during early stages of succession even before microbial crusts are visible (Schmidt et al. 2008, Freeman et al. 2009b); and since photosynthetically active radiation only penetrates several millimeters into soil (Ciani et al. 2005, Mitter 2013), these photoautotrophic communities are limited to the upper several millimeters of early successional soils. Likewise, microbial communities that depend on aeolian derived organic matter (Freeman et al. 2009a, Swan 1992, Mladenov et al. 2012) may also need to be near the soil surface to compete for access to these substrates. It is therefore not surprising that microbial assemblages found at or near the soil surface differ significantly from those found deeper in the soil during the pre-plant stage of primary succession following glacial retreat (Rime et al. 2015). However, bulk-sampling approaches common to studies of microbial succession mix vastly different community types together creating a homogenous sample and thus masking finer-scale community-level ecological characteristics.

The present study focuses on phototroph-dominated BSC communities that are colonizing the forefield of a retreating glacier (Midtre Lovénbreen) in the High Arctic (~79°N). As this glacier recedes, it is leaving behind a mosaic of geomorphological features (Glasser and Hambrey 2001, Hambrey et al. 2005), including 1-to-10-meter diameter patches of fine textured sub-glacially derived sediments known as glacial flour (Fig. 1). These sediment patches are initially water-saturated following glacier retreat resulting in the formation of a flat surface (with a texture reminiscent of agar) that is conducive to colonization by microorganisms. During early October 2022, we observed that these flat sediment patches near the terminus of Midtre Lovénbreen were being colonized by discrete microbial colonies that were green to black in color (Fig. 1 and Suppl. Fig. S1). We sampled an array of these small colonies as well as the bare sediment immediately adjacent (2 cm) to the colonies (Suppl. Fig. S1). This paper describes the molecular characterization of these discretely bounded BSC communities and the still bare sediments that they are colonizing. This novel comparative approach provides an opportunity to illuminate the earliest stages of primary succession at the microbial community level—in an ecosystem undergoing rapid environmental changes due to climate warming.

Methods

Study site and field sampling

Midtre Lovénbreen is a polythermal valley glacier in a north-facing catchment located 4–5 km SE of Ny-Ålesund research station (78° 53′ N, 11°59′ E), Svalbard (Suppl. Fig. S2). The dominant rock formations in the Midtre Lovénbreen catchment are conglomerations of felsic igneous fragments and meta-sediments, including carbonate rocks and coal seams (Harland et al. 1997). Mean annual air temperature at Ny-Ålesund has increased from −5.6°C (1993 to 2002) to −2.5°C (2013 to 2022), whereas mean annual precipitation has not increased in the last 30 years, averaging about 420 mm annually (Seklima 2022). In addition, soil temperatures increased by 3.6°C between 1998 and 2017 (Boike et al. 2018). The glacier has been receding since about 1890 and has left behind a mosaic of landforms, including patches of sub-glacial sediments, linear trains of supra-glacial debris, moraine mounds, small lakes, and braided outwash streams (Hambrey et al. 2005). The focus of the present study is the patches of formerly sub-glacial sediments (Fig. 1) near the receding front of Midtre Lovénbreen. Based on previous work, these sediments consist of ~25% clay, 40% silt, 35% sand, have a low cation exchange capacity (2.6 meq/100 g) and pH of about 8 (Cimpoiasu et al. 2024). More chemical and physical attributes of these sediments can be found in Cimpoiasu et al. (2024, 2025).

Two sediment patches were chosen for this study (Fig. 1) based on the presence of distinct colonies (0.5–1 cm in diameter) on the surface of the sediments in each patch. Patch 1 (78°53.7512 N, 12°03.5424 E) is 2.8 meters long (north to south) at its longest point and 2.1 m wide in the middle. Patch 2 is located 3.7 m to the ESE of patch 1 and is 2.0 m long (north to south) at its longest point and 2.1 m wide in the middle. The sediment patches had been uncovered by the glacier within the last 5–14 years (Cimpoiasu et al. 2024, Trejos-Espeleta et al. 2024).

On 4 October 2022, 6 intact microcolonies (~1 cm wide by 0.3 cm deep) from each sediment patch were lifted off the soil using a sterile spatula and placed in 2 ml sterile tubes. A similar sized piece of bare sediment was collected from 2 cm away from each microcolony, as controls. Three black colonies and three green colonies were sampled from each sediment patch, making a total of 12 sampled colonies and 12 controls. These samples were placed in a field cooler and transported by foot to the UK Arctic Research Station in Ny-Ålesund, Svalbard where they were frozen (–20°C) within 4 h of collection. The samples were transported frozen to the University of Colorado and kept frozen until they were processed as described below.

DNA sequencing and bioinformatics

A Qiagen PowerSoil DNA Extraction kit (Qiagen, Hilden, Germany) was used for extractions, and DNA concentration was quantified with a Qubit 3.0 Fluorometer. DNA was amplified (in triplicates) with 16S rRNA gene (515f-806r) for bacteria and archaea and 18S rRNA gene (1391f-EukBr) for eukaryotes. These 16S and 18S rRNA gene primers were chosen to allow us to directly compare our data to other recent studies of deglaciating terrain where these same primers were used (Hu et al. 2021, Solon et al. 2021, Vimercati et al. 2022, Trejos-Espeleta et al. 2024). Amplified triplicates were pooled and normalized to equimolar concentrations using a SequalPrep Normalization Plate Kit (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced in the Fierer Lab, University of Colorado, Boulder on an Illumina MiSeq setup for 16S rRNA gene amplicons (2×250 bp) and 18S rRNA gene amplicons (2×150 bp). Raw sequence reads were demultiplexed using idemp (https://github.com/yhwu/idemp), primers removed with cutadapt, v. 1.18 (Martin 2011), and amplicon sequence variants (ASVs) assigned with DADA2, v.1.26.0 (Callahan et al. 2016). Forward and reverse 16S rRNA gene reads were trimmed to 253 bp, while 18S rRNA gene forward and reverse were trimmed to 125 bp. ASVs were assigned taxonomy with the SILVA 138 database for 16S rRNA gene sequences (Quast et al. 2013) and the PR2 5.0 database for 18S rRNA gene sequences (Guillou et al. 2012). The mctoolsr R package was used to remove chloroplast and mitochondria ASVs from the 16S dataset as well as any misassigned sequences (e.g. bacteria) in the 18S rRNA gene dataset (Leff 2017). All sequences were deposited under NCBI BioProject PRJNA1180576, accession numbers SRR31184266 to SRR31184289 (16S rRNA ASVs) and SRR31184516 to SRR31184539 (18S rRNA ASVs).

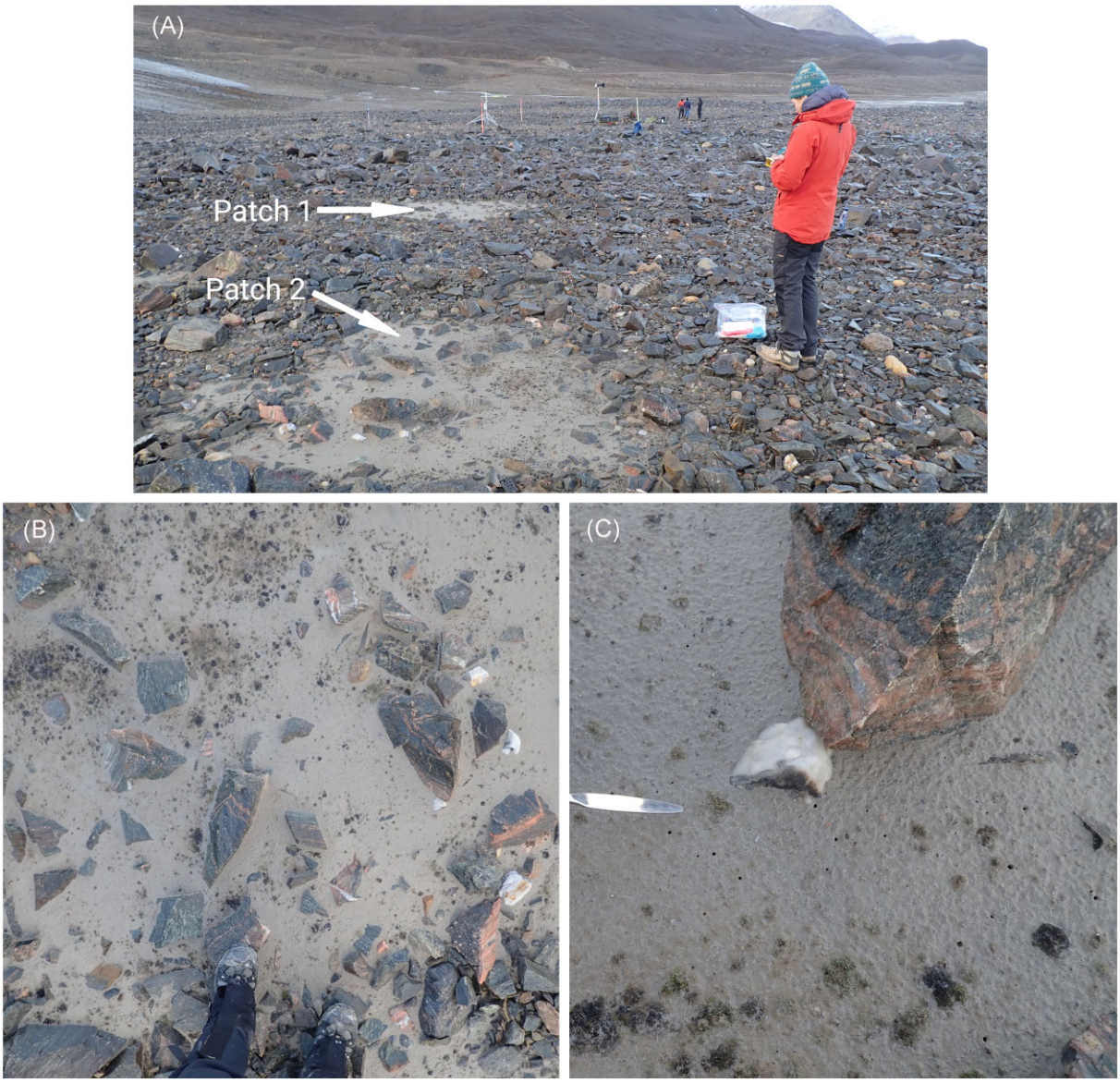

Figure 1. (A) The two sediment patches sampled for the present study; patch 2 is in foreground, and patch 1 is between patch 2 and instrument site 1, visible 40 m to the west (Cimpoiasu et al. 2024, 2025). Midtre Lovénbreen is to the south (left) of the patches. (B) Patch 2 with boots for scale at the south end (bottom) of the picture. (C) Spatula pointing at a colony that was sampled (sample P2-6A) for this study. Spatula is 7 mm wide at its widest point. Other colonies are also visible throughout the photo, and colonies can be seen merging with each other towards the bottom of the photo.

Table 1. Presumptive lichens based on the network analyses across all twelve pigmented colonies and on observations of high co-relative abundances of algae (e.g. ASV 13) and fungi (e.g. ASV 68) known to form lichens in other environments.

Photo-biont	Blast match (% match)	NCBI Acc. number (reference)	Myco- biont	Blast match (%)	NCBI Acc. number (reference)	Source
ASV_13 (18S)	*Chloroidium* (100%)	MH551496 (Darienko et al. 2018)	ASV_68	*Strigula* (96.12%)	MW375719 (Jiang et al. 2022)	Suppl. Fig. S3 P2.2A
ASV_296 (18S)	*Chloromonas* (93%)	LC683781 (Procházková et al. 2023)	ASV_68	*Strigula* (96.12%)	MW375719 (Jiang et al. 2022)	P1.5A
ASV_45 (16S)	Gloeobacteraceae (100%)	FJ977153 (Jungblut et al. 2010)	ASV_7	*Exophiala* (100%)	CP034379 (Schultzhaus 2019)	Fig. 6
ASV_924 (18S)	*Chlamydomonas* (100%)	AB290339 (Nakada and Nozaki 2007)	ASV_497	Ascomycota (94.6%)	AJ496252 (unpublished)	Fig. 6

NCBI accession numbers and references are for the closest BLAST match.

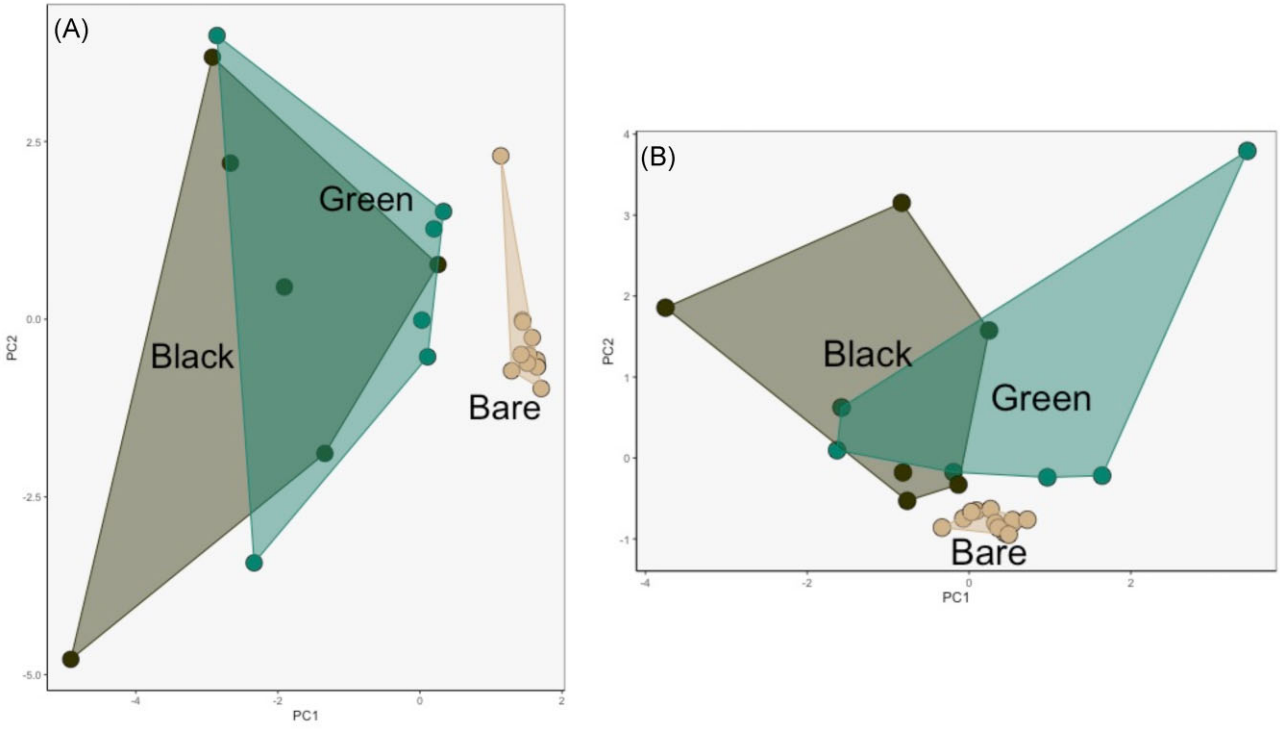

Figure 2. PCA plots of the 12 colonies (green and black) and the 12 controls (bare) for each colony. (A) is the eukaryotic (18S rRNA genes) communities, and (B) is the 16S rRNA communities. The green and black communities were significantly different from the bare soils for both the 18S and 16S rRNA communities ($P < .002$, PERMANOVA), but the green and black communities were not significantly different from each other. As is evident from the plots, the colonies also showed much higher community dispersion than the control (bare) sediments ($P < .001$ for 18S data, $P < .03$ for 16S data, PERMDISP).

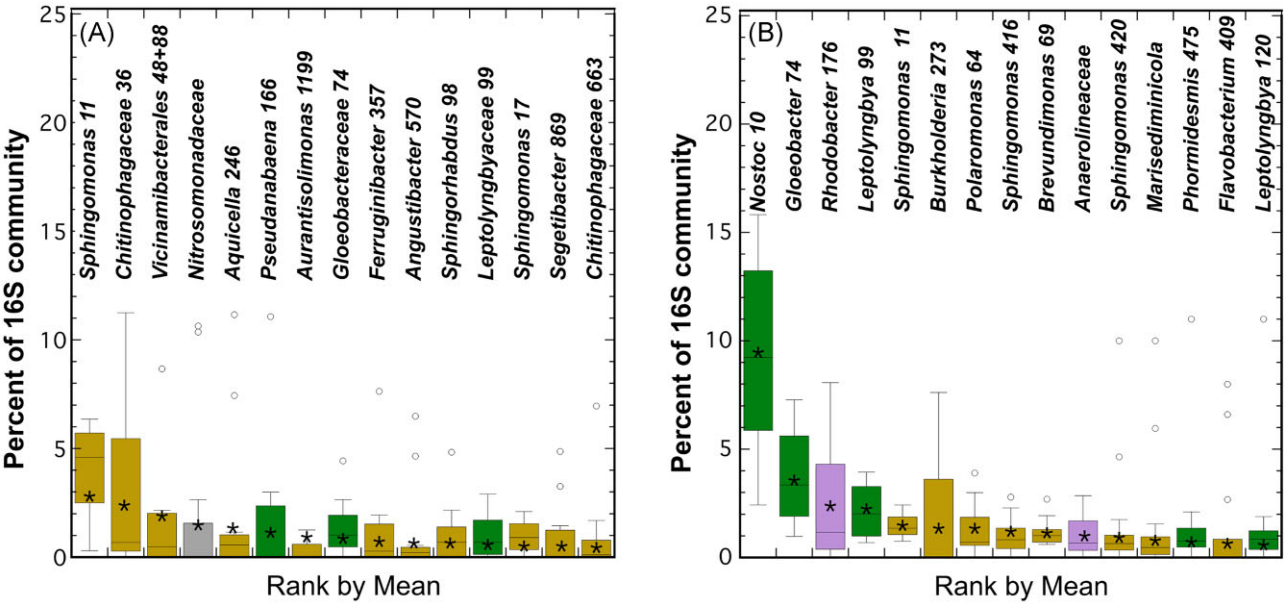

Figure 3. Rank abundance box plots of the top 15 16S ASVs in the control sediments (A) and the colonies (B). Asterisks (*) denote the mean, the boxes encompass the middle two quartiles of data, and the open circles are outliers. Green boxes are cyanobacteria, brown are heterotrophs, grey are chemoautotrophs, and purple are photoheterotrophs. The top 15 ASVs made up 30% and 26% of the total community for the colonies and the controls, respectively.

Community composition and richness

Differences in community composition between black, green, and control communities were determined with a permutational multivariate analysis of variance test (PERMANOVA) and a permutation test for homogeneity of multivariate dispersion (PERMDISP) (Anderson 2006, Anderson 2017). Prior to analyses, the robust center-log ratio transformation was applied to both 16S rRNA gene and 18S rRNA gene datasets to account for differences in library

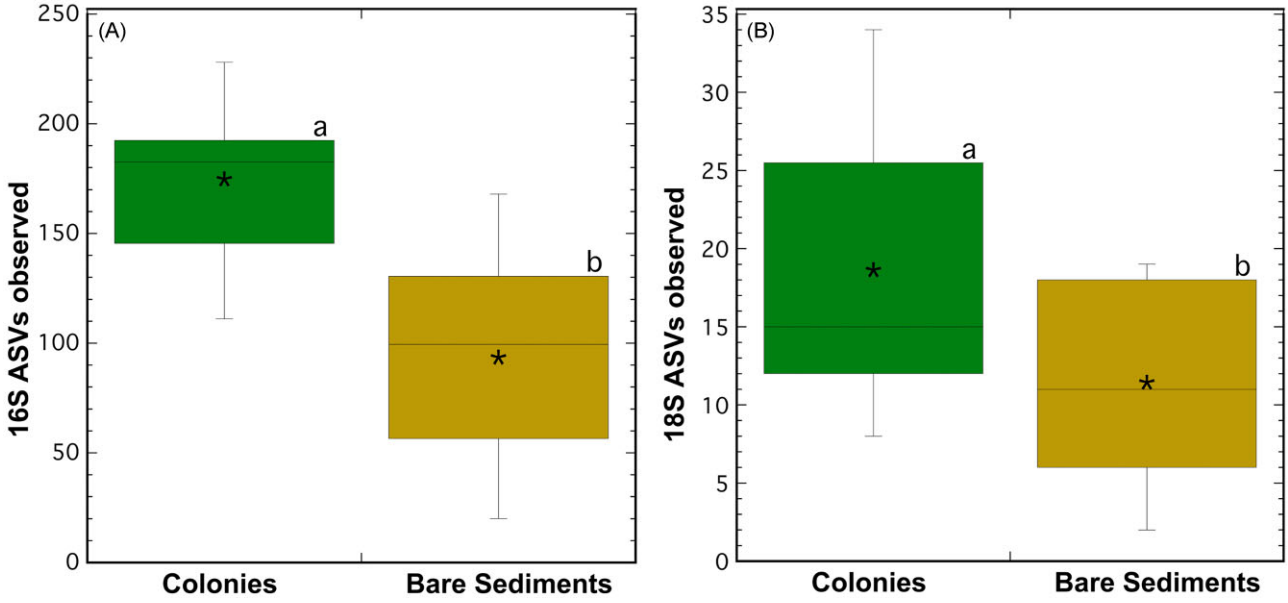

Figure 4. ASV richness for 16S rRNA gene (A) and 18S rRNA gene (B) rarefied data for the 12 colonies (green) and 12 control (brown) sediment samples. Asterisks (*) denote the mean, and different letters represent significant differences in pairwise comparisons (Welch t-tests, $P < .001$).

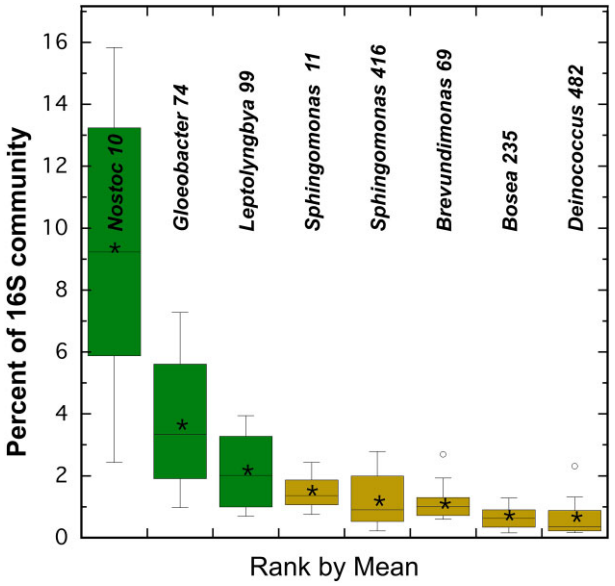

Figure 5. Rank abundance box plot of potential keystone species, that is, the only 16S ASVs that showed 100% prevalence (12 out of 12 colonies). Asterisks (*) denote the mean relative abundance for each ASV. Green boxes are cyanobacteria, and brown represents presumed heterotrophs in the Alphaproetobacteria (*Sphingomonas*, *Bosea*, and *Brevundimonas*) and Deinococcota (*Deinococcus*). In contrast, ASV 11 (*Sphingomonas* 11) was the only 16S ASV found in all 12 control (bare) samples (data not shown).

size (Martino et al. 2019). These tests were computed in the R programming language, v.4.2.3 (R Core Team 2021), with the vegan package, v.2.6.4 (Oksanen et al. 2024), and RVAideMemoire, v.0.9-83-7 (Herve 2023) package. Ordinations were generated with principal components analysis (PCA) in vegan and visualized with ggplot from the tidyverse (v.2.0.0) package (Wickham et al. 2019).

Differences in ASV richness among colonies and adjacent controls were tested with Welch t-tests. Before the t-tests, rarefaction by subsampling without replacement was performed to account for differences in library size (Schloss 2024). Data were rarefied with the phyloseq package, v1.48.0 (McMurdie and Holmes 2013), with a sequencing depth of 4231 as the cut-off for 16S and a depth of 133 for 18S data sets. Observed richness of ASVs (S) and Shannon diversity (H') were computed with the vegan package (v2.6.10), and Pielou evenness (J') was calculated as $J' = H'/\ln(S)$. Code for the above community analyses can be found at: https://github.com/adam-solon/Schmidt-et-al_in-review/.

Co-occurrence network analysis

Network analysis was performed using sparse inverse covariance estimation with the SPIEC-EASI R package (Kurtz et al. 2015). To simplify the communities and focus on the most relatively abundant taxa in the BSCs, we took the top 100 most relatively abundant ASVs from the 16S and 18S datasets, from just the BSC samples, and combined them into a concatenated dataset of read counts to input into the SPIEC-EASI program. The SPIEC-EASI program first performs a center log ratio transformation. We used the Meinshausen-Buhlmann's neighborhood selection method with 50 subsamples. To describe the topology of the resulting network, a set of measures (e.g. average node connectivity, average path length, clustering coefficient, etc.) were calculated. The networks were visualized using the Igraph R package (Csardi and Nepusz 2006). We visualized the complete network with 200 taxa (nodes), as well as a subset of that network with just positive relationships of among algae, cyanobacteria, and fungi, which could represent potential lichen associations. R code for the network analyses can be found at: https://github.com/cliffbueno/Svalbard.

Results

Community composition and richness

Community analyses of the entire data set (12 colonies and 12 adjacent controls) showed that colony color (black or green) and patch location (patch 1 or patch 2) did not determine phylogenetic structure of the colonies or controls. For example, as visualized by the spread of the data shown in Fig. 2, the bare (control) communities were tightly clustered and were significantly different from the colonies ($P < .002$ for both 16S and 18S data sets, PERMANOVA).

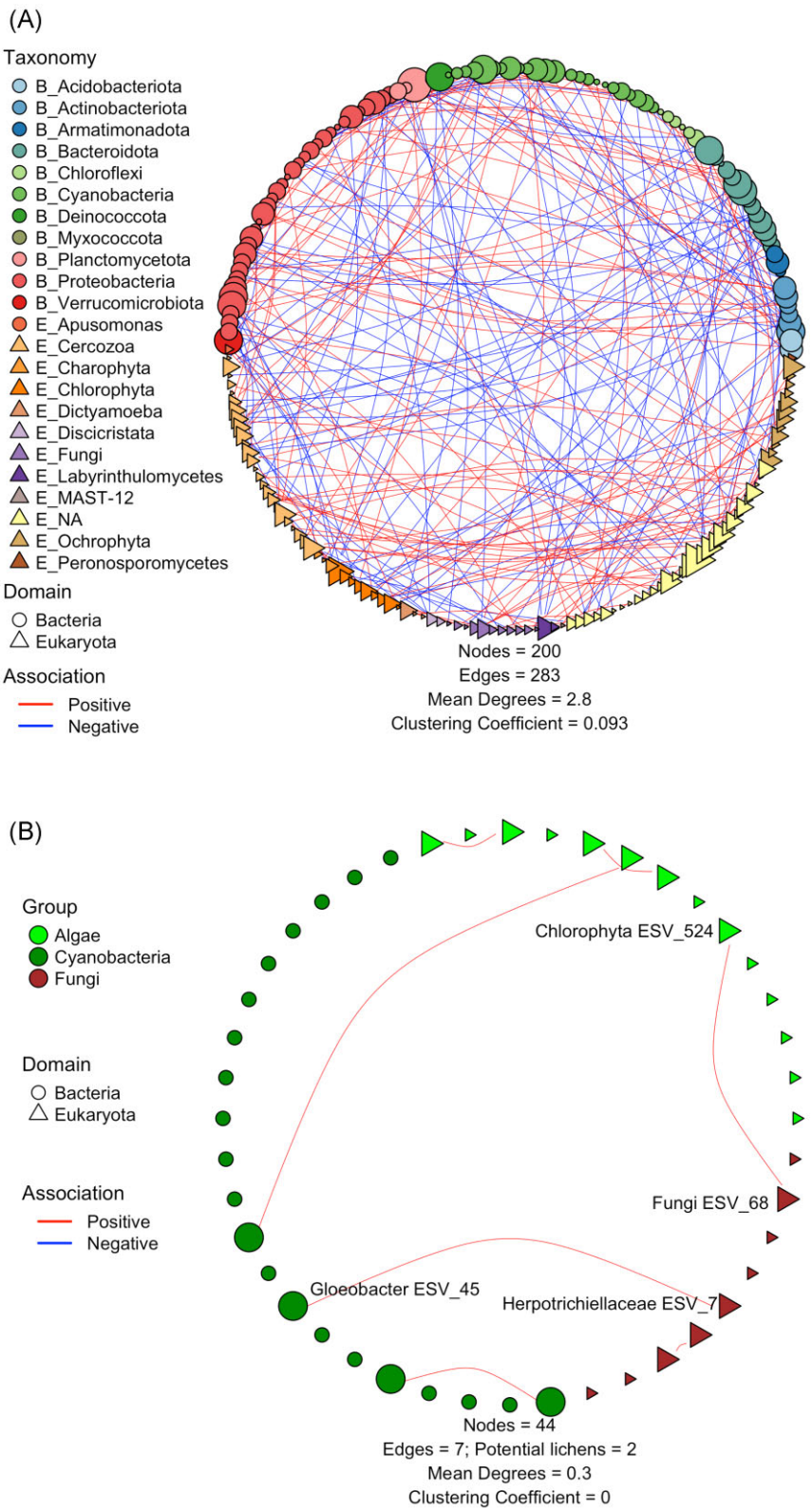

Figure 6. (A) ASV co-occurrence networks based on correlation analysis among 18S and 16S data sets for the colonies. Red lines are positive correlations, while blue lines are negative correlations. Each symbol represents an ASV, and the size is proportional to the number of connections. (B) Positive correlations among phototrophs and fungi (in all samples) possibly indicating the formation of lichen associations.

In contrast, the colonies showed an unexpectedly broad range of community types that did not correspond with colony color (Fig. 2) and had a significantly higher degree of community dispersion than the bare soils using a Permutation test for Homogeneity of Multivariate Dispersion (PERMDISP, $P < .001$ for 18S data, and $< .03$ for 16S data). Given these results, the rest of the analyses reported in this paper are based on pairwise comparisons of the 12 colonies to the 12 adjacent control samples regardless of color or location.

Taxonomic comparisons of the colonies to adjacent control sediments show higher overall richness and higher relative abundance of phototrophs in the colonies compared to the controls. For example, comparison of the top 15 16S ASVs shows a shift towards a community dominated by phototrophs in the colonies (Fig. 3, Suppl. Fig. S1), whereas the 18S community shifted more towards a dominance by fungi in the genus *Exophiala* (Herpotrichiellaceae, Eurotiomycetes) in the colonies compared to the control sediments (Suppl. Figs. S3, S4). Total ASV richness of rarefied data (Fig. 4) was significantly higher in the colonies for both the 16S and 18S rRNA gene data sets (Welch t-tests, $P < .001$). Shannon diversity for the 16S data was also significantly higher ($P < .02$) in the colonies (4.2, SE 0.05) than the bare sediments (3.7, SE 0.17), but not for the 18S data, where both had Shannon diversity of 1.6. Evenness of the 16S data for colonies and bare sediments were 0.81 and 0.83, respectively, whereas evenness for the 18S data was significantly higher ($P < .005$) in the bare sediments (0.85, SE 0.03) than the colonies (0.55, SE 0.06).

Keystone community members

To further understand which ASVs are key to community assembly of the microcolonies, we determined which were found in all twelve colonies (i.e. showed 100% prevalence). Surprisingly, there were only eight 16S ASVs (out of 1800 total 16S ASVs) that were found in all twelve colonies (Fig. 5), and all of these had a relative abundance of greater than 0.2% in every colony. The top 3 of these keystone colonizers were cyanobacteria, and the rest were presumptive heterotrophs in the Alphaproteobacteria and Deinoccaceae (Fig. 5). In contrast, only one 16S ASV (ASV_11, a *Sphingomonas*) was found in all twelve of the control (bare) samples.

Among eukaryotes (18S rRNA gene ASVs), only one phylotype was found in all 12 colonies, a diatom (ASV_146) related to the genus *Sellaphora* (which was also found in 6 of 12 bare controls). A fungus (*Exophiala* ASV_9) in the Herpotrichiellaceae was the only ASV that was more relatively abundant than ASV 146 in the bare soils, and it was present in 11 of the 12 control samples. Representatives of *Exophiala* (ASV_7 and ASV_9) were also the most relatively abundant eukaryotes in both the controls and colonies (Suppl. Fig. S4).

Cross-domain networks

An inter-domain network analysis of the twelve pigmented colonies was also done to determine if there were significant positive and negative interactions among the most relatively abundant 16S and 18S ASVs in the colonies. There were 283 within and cross domain associations (164 positive, 119 negative), with mean number of connections ("degrees") of 2.8 and a clustering coefficient of 0.093 (Fig. 6a). Taxa with the most connections and betweenness centrality (extent to which a node lies on paths between other nodes) included ASVs from the SAR supergroup (e.g. the diatom *Sellaphora*), and the green alga *Scherffelia*, and the Acidobacteriota genus *Blastocatella*. There were 2 potential lichen associations identified—one between the cyanobacterium *Gloeobacter* and a fungus in the Herpotrichiellaceae, and one between the alga *Chlamydomonas* and unidentified Ascomycete fungus (Fig. 6b, Table 1). Two other potential lichens were identified from observations of co-occurring dominant species, as shown in Suppl. Fig. S3.

Discussion

The work presented here is the result of the serendipitous discovery of discrete, spatially independent, early successional microbial biocrust colonies forming on the foreland of a retreating glacier (Midtre Lovénbreen) in the High Arctic. This glacial forefield has been studied for many decades and has yielded key insights into broad patterns of primary succession of plants, animals, and microbes in the High Arctic (e.g. Hodkinson et al. 2003, Schütte et al. 2009, Borin et al. 2010, Bradley et al. 2016, Kim et al. 2022, Pedersen et al. 2022, Trejos-Espeleta et al. 2024). The present study builds on this foundation by focusing on the early stages of BSC formation and ecosystem development by sampling individual BSC colonies before they coalesce to form a continuous soil crust.

An important takeaway from this study is that the microcolonies observed in the field are complex communities dominated by phototrophs that are supporting a diverse community of saprophytic (e.g. fungi) and predatory heterotrophs (e.g. *Rhogostoma*) (Suppl. Fig. S4). In contrast, the bare (control) sediments just 2 cm outside the colonies host microbial assemblages dominated by heterotrophs (e.g. Fig 3, Suppl. Figs. S1, S3, S4) that are significantly different than the BSC communities (Figs. 2, 4). In addition, the degree of community dispersion among the control (bare) communities was also significantly lower ($P < .001$ for 18S, $P < .03$ for 16S data) compared to the wide variety of community types in the colonies (Fig. 2). This finding was not expected and indicates that there are many different types of early successional BSCs and a high degree of stochasticity in the initial stages of BSC formation. This stochasticity may be driven by which microbes arrive first (priority effects) at each colony microsite, by microscale variations in abiotic factors underlying where microcolonies can form, or by variation in unknown allogenic factors (Wojcik et al. 2021). Whatever the mechanism, these results are the first evidence for high heterogeneity of BSC community types assembling on recently deglaciated terrain in the High Arctic, or indeed in any ecosystem; a finding that was only revealed due to the directed, fine-scale sampling of individual colonies done for this study.

Despite the high degree of community level differences among the twelve colonies, there were eight highly abundant bacterial ASVs found in all twelve colonies (Fig. 5), indicating that they may be integral or keystone species for the formation of early successional communities in this ecosystem. Not surprisingly, the most relatively abundant of these prevalent ASVs were phototrophic bacteria (Fig. 5), as has been reported for many glacial forefields in extreme polar and high-elevation environments (Hodkinson et al. 2003, Schmidt et al. 2011, Schmidt et al. 2017). The most relatively abundant organism (ASV 10) was a 100% match to several N-fixing *Nostoc* isolates, confirming that N-fixation is an important trait needed for the establishment of early successional colonies in nutrient-limited glacial forefields (Schmidt et al. 2008, Knelman et al. 2021). The prevalence of ASV 99 (Leptolyngbyaceae) was also not surprising and is a 100% match to other High-Arctic cyanobacteria (Harding et al. 2011), including a previously reported sequence from the Midtre Lovénbreen forefield (Borin et al. 2010).

More surprising was the high prevalence and relative abundance of ASVs that best matched cyanobacteria in the Gloeobacterales (Figs. 3, 5), an enigmatic, deeply divergent group of non-filamentous cyanobacteria that lack thylakoids (Rahmatpour et al. 2021). Until recently, Gloeobacterales were not known to be important in cold systems, but our finding is backed up by recent reports from the Arctic and Antarctica (Pessi 2017, Grettenberger et al. 2020), including in soil crusts from Svalbard (Pushkareva et al. 2015). However, the presence of Gloeobacterales very early in succession has not been previously documented. The network analyses (Fig. 6) also indicated that ASV 45 (Gloeobacteraceae) significantly co-occurred with fungal ASV 7 (*Exophiala*, Herpotrichiellaceae) indicating that the two may be symbiotically associated, perhaps as a lichen (Table 1). However, lichen associations between members of Herpotrichiellaceae and Gloeobacteraceae have not been previously reported, and our work suggests that further exploration of potential lichen pairings is warranted along post glacial chronosequences. Involvement in lichens could also help explain why the Herpotrichiellaceae are the dominant fungal group found in this study and studies of the earliest stages of succession following glacial retreat at elevations above 5000 meters in the High-Andes (Hu et al. 2021), but no direct evidence for these hypothesized lichens currently exists.

A more plausible hypothesized lichen pairing revealed in the present study is that between algae in the genus *Chloroidium* and fungi in the Strigulaceae (Suppl. Fig. S3, Table 1). Members of both groups have been shown to form lichens in non-polar environments (Darienko et al. 2018, Vančurová et al. 2021, Jiang et al. 2022), but there are no known reports of either of them forming lichens in polar soils. Proving that these two organisms can form a lichen in polar soils will require even more focused sampling of early successional biocrust than was done for the current study and could shed new insights on how quickly soil lichens can form following glacial retreat.

Overall, the present study demonstrates a new approach to studying how microbial communities assemble and function during the important early stages of primary succession following glacial retreat. As with any new approach to studying complex ecological processes, this approach raised more questions than it answered. Nonetheless, this study yielded several important insights into how microbial communities assemble, including (1) the unexpected diversity and high community dispersion of phototrophic colony types forming on newly de-glaciated sediments; (2) the identity of keystone cyanobacteria that are likely needed for successful colony formation; and (3) predictions of previously unknown lichen pairings that may be important during the early stages of ecosystem development. These insights would not have come to light if this study had depended on bulk sampling approaches most often used in soil ecological studies. Future work will include following the growth and eventual merging of individual colonies in the field and comparing the genetic makeup of Midtre Lovénbreen colonies to similar ones recently discovered on other glacier forefields on Svalbard and elsewhere.

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Supplementary data

Supplementary data is available at *FEMSMC Journal* online.

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