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Research Article

Dynamic trophic shifts in bacterial and eukaryotic communities during the first 30 years of microbial succession following retreat of an Antarctic glacier

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Abstract

We examined microbial succession along a glacier forefront in the Antarctic Peninsula representing ~30 years of deglaciation to contrast bacterial and eukaryotic successional dynamics and abiotic drivers of community assembly using sequencing and soil properties. Microbial communities changed most rapidly early along the chronosequence, and co-occurrence network analysis showed the most complex topology at the earliest stage. Initial microbial communities were dominated by microorganisms derived from the glacial environment, whereas later stages hosted a mixed community of taxa associated with soils. Eukaryotes became increasingly dominated by Cercozoa, particularly Vampyrellidae, indicating a previously unappreciated role for cercozoan predators during early stages of primary succession. Chlorophytes and Charophytes (rather than cyanobacteria) were the dominant primary producers and there was a spatio-temporal sequence in which major groups became abundant succeeding from simple ice Chlorophytes to Ochrophytes and Bryophytes. Time since deglaciation and pH were the main abiotic drivers structuring both bacterial and eukaryotic communities. Determinism was the dominant assembly mechanism for Bacteria, while the balance between stochastic/deterministic processes in eukaryotes varied along the distance from the glacier front. This study provides new insights into the unexpected dynamic changes and interactions across multiple trophic groups during primary succession in a rapidly changing polar ecosystem.

Keywords: Antarctic Peninsula, Cercozoa, community-assembly mechanisms, eukaryotic primary producers, glacier chronosequence, microbial succession, network analysis

Introduction

Understanding the successional patterns of organisms over space and time has been a long-standing research topic in ecology. Now it is more important than ever, as accelerating glacier recession is a phenomenon that is affecting most polar and high-elevation areas worldwide (Haeberli et al. 2007, Yao et al. 2012). New terrestrial landscapes that emerge from underneath the ice provide new niches for colonizing microorganisms that face dramatic changes in environmental conditions, transitioning from subglacial often anoxic conditions (Tranter et al. 2002, Wynn et al. 2006, 2007) to periglacial oxic conditions, increased nutrient availability and changes in soil temperature (Schmidt et al. 2008, Schütte et al. 2009). Ongoing climate change coupled with changes in global biogeochemical patterns (Galloway et al. 2008) make it important to understand microbial successional dynamics in these changing landscapes; however, studies in these systems are still lagging in Antarctic regions.

Chronosequences in recently deglaciated areas represent ideal sites to study successional trajectories of microbial communities (Nemergut et al. 2007) and allow for detailed examinations through space-for-time substitutions as a proxy for time since deglaciation (Matthews 1992, Walker et al. 2010). Concurrent examinations of bacterial and eukaryotic phyla have often been overlooked as most studies have focused on either Bacteria alone (Schütte et al. 2009, Philippot et al. 2011, Bajerski and Wagner 2013, Khan et al. 2020) or Bacteria simultaneously with fungi or algae (Jiang et al. 2018, Franzetti et al. 2020), ignoring potentially important protist communities (Oliverio et al. 2020). Microbial diversity patterns are not consistent in different glacial chronosequences, and these differences have been attributed to heterogeneity in soil structure, nutrient levels, and environmental conditions (Bradley et al. 2014). Moreover, most chronosequences investigated span tens of years or even centuries (Jangid et al. 2013, Kazemi et al. 2016) and fail to capture the successional patterns and dynamics within the earliest stages of succession, where they are predicted to change most rapidly (Sigler et al. 2002). Several environmental factors, such as pH, moisture, carbon, nitrogen, and phosphorous contents, drive soil microbial community succession in deglaciated soils (Zumsteg et al. 2012, Jiang et al. 2018, Khan et al. 2019, Garrido-Benavent et al. 2020), but differences

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in drivers of bacterial and eukaryotic succession remain unclear. These edaphic properties have been shown to influence soil microbial community composition in general, not just in successional contexts (Tedersoo et al. 2014, Fierer 2017). Furthermore, dispersal limitation and stochasticity can play a role in microbial community assembly, and differences in the relative importance of these factors between bacteria and eukaryotes could potentially drive differences in their successional patterns (Nemergut et al. 2013, Chen et al. 2020).

Microbial communities play a key role in primary succession and pedogenesis in recently deglaciated soils (Lazzaro et al. 2009, Schütte et al. 2009) and help with the establishment of plants (Bradley et al. 2014). A complete model of colonization patterns and dynamics remains elusive due to the heterogeneity of proglacial landscapes and different temporal and spatial resolutions of the investigated chronosequences. A number of studies have shown that bacterial and eukaryotic communities have contrasting community assembly patterns along glacier forefield transects and respond differently to environmental variables (Brown and Jumpponen 2014, Jiang et al. 2018, Khan et al. 2019). Stochastic processes are expected to be more important in structuring early-stage successional communities, especially for Bacteria, while deterministic processes are potentially more relevant in structuring communities in later stages (Ferrenberg et al. 2013, Brown and Jumpponen 2014). In addition, deterministic processes are expected to mediate bacterial community succession (Schmidt et al. 2014), while eukaryotic communities, especially fungal communities, are more influenced by stochastic processes (Jiang et al. 2018). Deterministic models establish that successional changes are directional, with dissimilarities among communities decreasing over time (Clark 2009). On the other hand, stochastically assembled communities are assembled by probabilistic dispersal, ecological drift, or historical inertia (Jetschke and Hubbell 2002).

Recent work has also examined microbial co-occurrence networks across successional gradients (Dini-Andreote et al. 2014, Farrer et al. 2019, Dong et al. 2022). Macroorganisms typically show that network complexity tends to increase across succession because of the parallel increase in resources (Neutel et al. 2007), but microbial studies found that network complexity was highest early in the succession, possibly because great dynamism in environmental conditions at initial stages of succession may promote the emergence of multiple niches (Dini-Andreote et al. 2014). Community network structure has important implications for resilience (Mandakovic et al. 2018) and stability (Neutel et al. 2007) and microbial co-occurrence networks shed light on how taxa potentially interact with each other across spatial and temporal gradients and help identify habitat affinities or shared physiologies (Barberán et al. 2012).

The Antarctic Peninsula is characterized by cold-maritime climatic conditions (Vieira and Ramos 2003) and has been strongly affected by rapid warming in the last few decades (Cook et al. 2005, Turner et al. 2014), driving the expansion of deglaciated areas (Lee et al. 2017). A total of 87% of glaciers along the west coast of the Antarctic Peninsula have receded over the last half century (Cook et al. 2005), a trend which is expected to continue. We examined a soil chronosequence of a glacier forefield located near the US Antarctic Palmer Station. The overall objective of this study was to simultaneously analyze the succession of glacier forefield soil bacterial and eukaryotic communities as well as the influence of environmental factors underlying these patterns. Through Illumina amplicon sequencing we assessed the diversity, structure, and temporal dynamics along the glacier chronosequence in both Bacteria and Eukarya, as well as fungi and algae independently. We also examined the co-occurrence networks of Bacteria and Eukarya along the chronosequence. Additionally, we measured microbial biomass production via ^{3}H-leucine incorporation at different temperatures in soil samples from different stages of the chronosequence to monitor the short-term effect of increasing temperature in community successions along the soil gradient.

We hypothesized that

(1) Bacterial and eukaryotic communities would have contrasting assembly patterns along the soil chronosequence and would be driven by different environmental variables given their different roles in biogeochemical cycles, dispersion ability, and adaptation strategies.
(2) Determinism would be more relevant in structuring community succession in Bacteria than in Eukarya, while stochasticity would be more important for both communities in the early stages of succession.
(3) Network complexity would be highest early in the succession, possibly because strong environmental dynamics associated with a changing environment elicit high phylotype coexistence.
(4) Microbial production would increase both with distance from the glacier front and with higher incubation temperatures, with stronger temperature effects later in the chronosequence.

Methods

Field site and sampling approach

The study site is located on Anvers Island off the west coast of the Antarctic Peninsula, just to the east of Palmer Station (64° 46 S, 64° 04 W) on a gently sloping terrace. The Marr Ice Piedmont that covers much of Anvers Island extends to this area. Based on satellite and aerial images and observations by Palmer Station personnel, this area appears to have started becoming ice-free around 1963, and we estimate that the glacier has retreated at an average rate of 10 m/year since that time. Our transect extends 300 m from the edge of the glacier, representing a soil chronosequence of approximately 30 years (Fig. 1). A total of 27 samples were collected during January 2013 along a transect at increasing distances from the glacier front (0, 20, 40, 80, 100, 120, 160, 180, 200, 260, 280, and 300 m). A total of two to three replicates were collected for each distance category. The studied landscape is virtually devoid of vascular plants and all samples were collected from soils that did not contain any visible plant material within at least 1 m in all directions. Each sample was collected using a sterile spatula and contained 50–100 g of the upper 4 cm of soil within an area of $\sim$ 100 cm^2. Soil samples were divided into three main stages for analysis: "Early" with samples from 0, 20, and 40 m; "Mid" with samples from 80, 100, 120, and 160 m; and "Late" with samples from 260, 280, and 300 m. Samples were stored at −20°C before being shipped to the University of Colorado at Boulder, where they were kept at −80°C for long-term storage.

Geochemical analysis

Carbon and nitrogen concentration and isotopic analyses were performed in the Earth System Evolution Lab (EaSEL) at Iowa State University following Johnson et al. (2017), after freeze-drying at the University of Colorado Boulder. Samples were freeze-dried for 24 h. Subsequently, dried samples (~1–5 g) were crushed to a powder in an agate ball-mill for approximately 1 min. Agate container and balls were cleaned with deionized (DI) water and ethanol in between each sample. A volume of 5 ml of 10% HCl was added to each sample in centrifuge tubes to remove carbonate minerals.

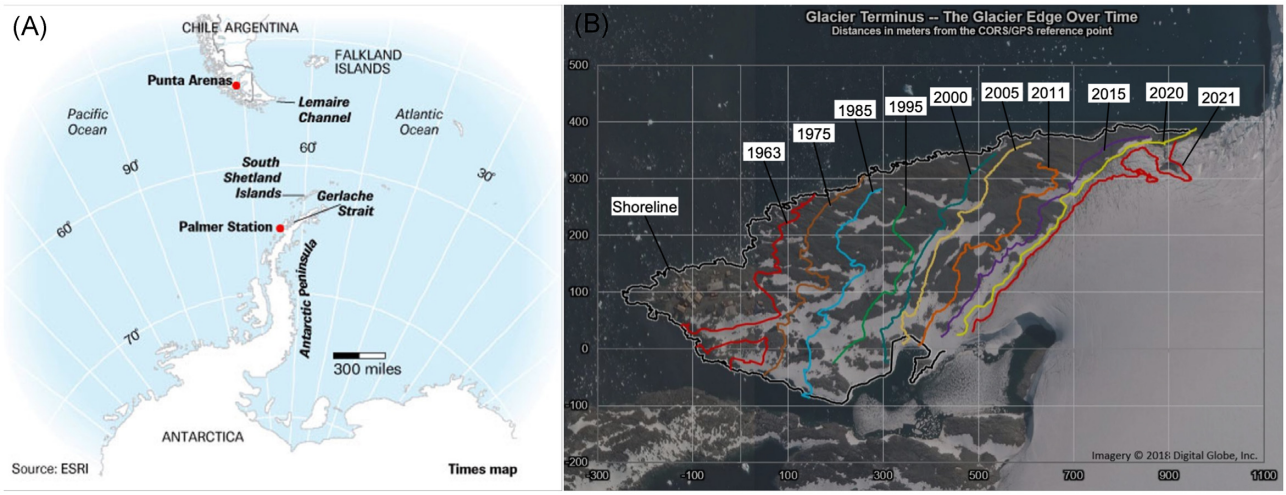

Figure 1. Overview of the glacier chronosequence. **(A)** The soil chronosequence is located on the Anvers Island, off the west coast of the Antarctic Peninsula, next to the Palmer Station (64° 46 S, 64° 04 W). **(B)** Data on glacier terminus lines up to 2021 were collected via surveys with the UNAVCO Trimble R7 roving system, in conjunction with the UNAVCO PAL2 Base Station (Trimble NetR8).

Acidified samples sat in a sonicator overnight, after which they were centrifuged for 5 min. After decanting the acid waste, 5 ml of DI water were added to samples, vials were capped and shaken, and samples centrifuged again for 5 min. Water was decanted, and this rinse step was repeated two more times. Samples then dried at 70°C overnight.

Concentration and isotope ratios of carbon (C) and nitrogen (N) were determined on a Thermo IsoLink Flash Elemental Analyzer coupled to a Delta V Plus isotope ratio mass spectrometer. Between 10 and 40 mg of sample was weighed out into 9 × 5 mm tin capsules, and flash combusted at 1020°C with an excess of oxygen (300 ml/min for 4 s). A helium stream (140 ml/min) carried combustion products over a series of reagents (cobaltous oxide, reduced copper) to convert NO_x and CO species to N_2 and CO_2, respectively. Samples were compared against a series of standards, including external standards USGS62 (caffeine, $\delta^{15}N = 20.17‰$, $\delta^{13}C = -14.79‰$) and USGS42 (Tibetan human hair, $\delta^{15}N = 8.05 \pm 0.1‰$, $\delta^{13}C = -21.09 \pm 0.1‰$) as well as internal standards: peat ($\delta^{15}N = 4.24 \pm 0.1$, $\delta^{13}C = -28.41 \pm 0.1‰$) and urea ($\delta^{15}N = -2.52 \pm 0.1‰$, $\delta^{13}C = -37.05 \pm 0.1‰$). Analytical precision is 0.1‰, and reproducibility is 0.3‰ based on repeat analysis of standards and samples. We also monitored for blank contribution by running empty tin capsules and subtracting any influence from isotope and concentration values. Data are expressed in delta notation (δ = [R sample /R standard–1] · 1000), where R = $^{13}C/^{12}C$, or $^{15}N/^{14}N$. The standard is VPDB for $\delta^{13}C$ and atmospheric air for $\delta^{15}N$.

DNA extraction and sequencing

Total DNA was isolated from 0.5 g of homogenized soil using a PowerSoil® DNA Isolation Kit (MoBio Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. We assessed DNA quality and quantity by using the Qubit version 2.0 fluorometer (Qubit, London, United Kingdom) with the Qubit dsDNA HS assay kit (Thermo Fisher Scientific). For the amplification of the bacterial 16S rRNA gene, we used the oligonucleotide primer set 515F/806R (Caporaso et al. 2012), while for the amplification of the eukaryotic 18S rRNA gene, we used the Euk1391f/EukBr primer set (Amaral-Zettler et al. 2009; Earth Microbiome Project, accessible at http://www.earthmicrobiome.org/). Amplified DNA was pooled, normalized to equimolar concentrations using SequalPrep Normalization Plate Kits (Invitrogen Corp., Carlsbad, CA, USA), barcoded and then sequenced using an Illumina MiSeq (2 × 150 bp chemistry) at the BioFrontiers Sequencing Core Facility at the University of Colorado at Boulder. Sequences have been deposited in the NCBI SRA database under project PRJNA836937.

Sequence processing, ASV identification, and diversity analysis

Sequenced data were processed by first demultiplexing the data using idemp (https://github.com/yhwu/idemp) and trimming the primers with Cutadapt (Martin 2011). Quality plots obtained were visually inspected and 16S rRNA forward and reverse reads were trimmed to 145 bp, while 18S rRNA forward and reverse reads were trimmed to 120 bp. We used the DADA2 pipeline (Callahan et al. 2016) to infer Amplicon Sequence Variants (ASVs) that differ from each other at least by a single nucleotide and then to merge the paired-end reads and remove chimeras and singletons. Taxonomy was assigned against the Silva 132 NR99 reference database (Quast et al. 2013) and taxonomic assignments of the most abundant ASVs from each sample were verified by using BLAST (NCBI online tool). The "mctoolsr" R package was used to filter out chloroplasts, mitochondria, and eukaryotic reads from the 16S rRNA data and bacterial reads from the 18S rRNA data (Leff 2017). Among 18S rRNA data, fungal and algal reads were subset from total Eukarya sequences using the "filter_taxa_from_input" function in the "mctoolsr" R package and analyzed separately. Search terms for taxa to keep at SILVA level 1 were "Fungi" and "Chytridiomycota" for fungal reads and "Chloroplastida" and "Ochrophyta" for algal reads, respectively. ASV tables were rarified to the number of sequences in the lowest populated sample (7756 for 16S rRNA and 3845 for 18S r RNA). One sample was removed from the 18S rRNA dataset because of low number of reads.

Alpha diversity metrics [ASVs richness, Faith's phylogenetic diversity (Faith's PD; Faith 1992) and Pielou's Evenness] were calculated from the rarefied community matrices.

We calculated beta diversity using the weighted UniFrac distance metric (Lozupone and Knight 2005) implemented in the "phyloseq" R package (McMurdie and Holmes 2013), and Bray–Curtis dissimilarity implemented in the "mctoolsr" R package (Leff 2017). Principal Coordinates Analysis (PCoA) ordinations were

computed on UniFrac dissimilarities to illustrate differences in the phylogenetic composition of microbial communities across successional stages. To generate statistical support for these differences we ran the nonparametric PERMANOVA test (Anderson 2001) with 999 permutations with the "adonis" function in the "vegan" R package (Oksanen et al. 2019). Turnover rates per year were calculated dividing the Bray–Curtis dissimilarity index between consecutive succession stages by the difference in estimated soil age (years) between them (Wu et al. 2012). To evaluate whether samples differed in their dispersion, an analysis of multivariate homogeneity (PERMDISP; Anderson 2006) was run using the function "betadisper" with the "vegan" R package using default parameters. Hierarchical clustering analysis was performed on the UniFrac distance matrix using the "hclust" function using average linkage. The "cutree" function was used to identify distinct clusters within the dataset. The gradient of successional time and vectors of environmental variables were fitted onto the ordination space using the "envfit" function in the "vegan" R package. Variation partitioning analysis, performed with "varpart" in the "vegan" R package, was applied to show the individual contribution of each environmental factor (deglaciation age, moisture, soil pH, Total Organic Carbon (TOC), Total Organic Nitrogen (TON), and Carbon to Nitrogen (C/N) ratio) to the UniFrac dissimilarity in bacterial and eukaryotic communities along the chronosequence.

Beta diversity was further analyzed to quantify the turnover (ASV replacement) and nestedness (ASV loss) along the three-stage chronosequence for both Bacteria and Eukarya with the function "beta.multi" of the "betapart" R package using the Sørensen dissimilarity index (Baselga and Orme 2012, Baselga et al. 2018). To examine phylogenetic structure within communities we used the nearest taxon index (NTI; Webb et al. 2002), calculated with the "NTI.p" function in the "iCAMP" R package (Ning et al 2020). NTI is a standardized measure of the phylogenetic distance to the nearest taxon for each taxon in a sample. If NTI > 2 or < -2 deterministic processes are governing community assembly while if $-2 <$ NTI < 2 stochastic processes are at work (Stegen et al. 2012, Dini-Andreote et al. 2015).

To identify ASVs that were driving differences in composition among different chronosequence stages we performed a similarity percentage analysis (SIMPER) using the "vegan" R package.

Taxa responses

To test whether individual taxa exhibited spatial patterns, regressions between taxon relative abundance and distance from the glacier terminus were calculated in R using the "lm" function of the "stats" R package and corrected for multiple comparisons using the Bonferroni correction.

Network analysis

Co-occurrence network analysis in this study was performed for each chronosequence stage ("Early," "Mid," and "Late") for both Bacteria and Eukarya. Pairwise correlations between most abundant ASVs (200 for Bacteria and 100 for Eukarya) were calculated using Spearman's correlation based on the relative abundance. Only strong (Spearman's $\rho > |0.8|$) correlations were considered indicative of co-occurrence. The nodes in the networks represent individual ASVs and edges indicate that the correlation between the connected taxa is Spearman's $\rho > |0.8|$. In order to describe the topology of the resulting network, a set of measures (average node connectivity, average path length, degree distribution, clustering coefficient, modularity, betweenness centrality, and number of positive and negative correlations) were calculated (Newman 2003). Statistical analyses were carried out and networks visualized using the "igraph" R package (Csardi and Nepusz 2006). ASVs with the highest betweenness centrality in the networks are considered keystone taxa (Martín González et al. 2010, Vick-Majors et al. 2014, Banerjee et al. 2016, Choe et al. 2021).

Microbial community response to different incubation temperatures

To monitor the short-term effect of increased temperatures on soil microbial communities along the chronosequence, we incubated soil samples immediately following sampling from different distances (0, 40, 100, 160, 240, and 300 m) at five different temperatures (0, 5, 10, 15, and 20°C) for 24 h. Incubation temperatures of 0 and 5°C were chosen according to seasonal variation (Smith et al. 1995) while 10, 15, and 20°C were selected according to the predicted warming trend at the site and to investigate how a dramatic increase in soil temperature may affect short-term microbial response at different distances from the glacier front. Microbial biomass production was measured as ^{3}H-leucine incorporation following a modified procedure for soils from the protocol described in Ducklow et al. (2012).

Results

Bacterial community

General indicators of bacterial diversity, including ASV richness and Faith's PD increased significantly ($P < .05$) from "Early" to "Mid" sites along the chronosequence but did not increase form "Mid" to "Late" sites (Fig. 2A), whereas evenness did not increase over distance from the glacial terminus ($P = .28$, $F = 1.343$).

Bacterial community structure differed significantly among the three successional stages (PERMANOVA $P = .001$; $R^2 = 0.48$). Individual replicates in the earliest successional stage were less clustered than those observed for the "Mid" and "Late" soil stages (Fig. 3A), indicating higher phylogenetic turnover in species composition at the earliest stage. "Early" soil communities clustered separately from the rest of the succession and the earliest samples (0 m) clustered on their own (Fig. 3; Figure S1a, Supporting Information). Variation in bacterial communities within each successional stage was homogenous (betadisper $P > .05$). "Mid" and "Late" successional communities were more similar to one another (mean Bray–Curtis dissimilarity 0.6699) than to "Early" successional community (mean Bray–Curtis dissimilarity 0.848 and 0.911, respectively). The degree of variation in ASV composition along the chronosequence (β_{SOR}) and the turnover (β_{SIM}) component were comparable in the three stages of the chronosequence, whereas nestedness (β_{SNE}) was highest in the "Early" stage (Table 1). Overall, 85.8% of all ASVs were stage-specific, with "Early" stage having the highest number of stage-specific ASVs (Fig. 4A). Turnover rates according to the Bray–Curtis dissimilarity index significantly decreased with increasing distance from the glacier ($P = .034$) and the highest turnover rate per year occurred between the samples closest to the glacier front (0–20 m; Table 2). NTI values for each chronosequence stage were > 2 (Fig. 5A) suggesting that bacterial assemblages are shaped in a deterministic fashion across the whole chronosequence.

According to SIMPER analysis, five ASVs comprising 13.4% of the total relative abundance accounted for most of the community compositional differences among chronosequence stages (Fig. 3A). The top five ASVs that explained the most variance between each stage pair were identified as *Rhodoferax* sp. (Betaproteobacteria), *Novosphingobium* sp. (Alphaproteobacteria), *Nitrotoga*

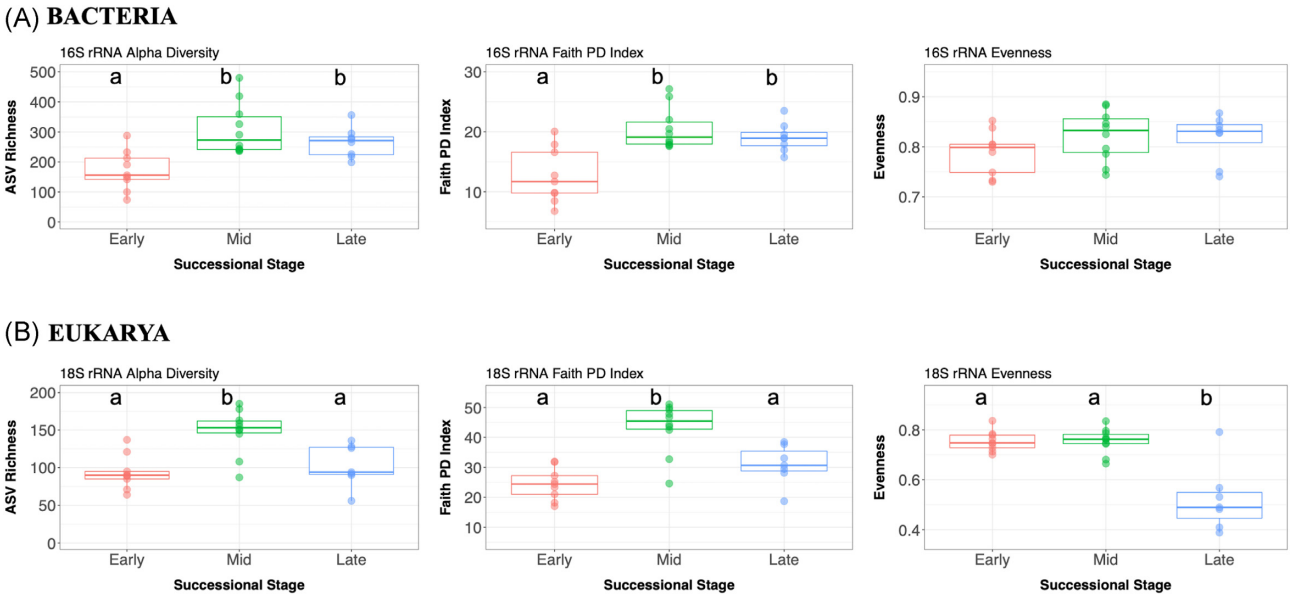

Figure 2. Bacterial and eukaryotic diversity metrics. Time series of diversity metrics of bacterial **(A)** and eukaryotic **(B)** communities (ASV richness, PD diversity, and evenness). Distance from glacier terminus has a significant effect on bacterial and eukaryotic ASV richness ($P = .001$, $F = 9.173$ and $P = .001$, $F = 11.89$, respectively) and Faith's PD ($P < .001$, $F = 12.32$ and $P < .001$, $F = 19.57$, respectively). It does not affect bacterial evenness ($P > .05$) but affects eukaryotic evenness ($P < .001$, $F = 21.38$). Results of Tukey *post hoc* tests for comparing multiple treatments are shown as letters, where different letters represent significant pairwise differences.

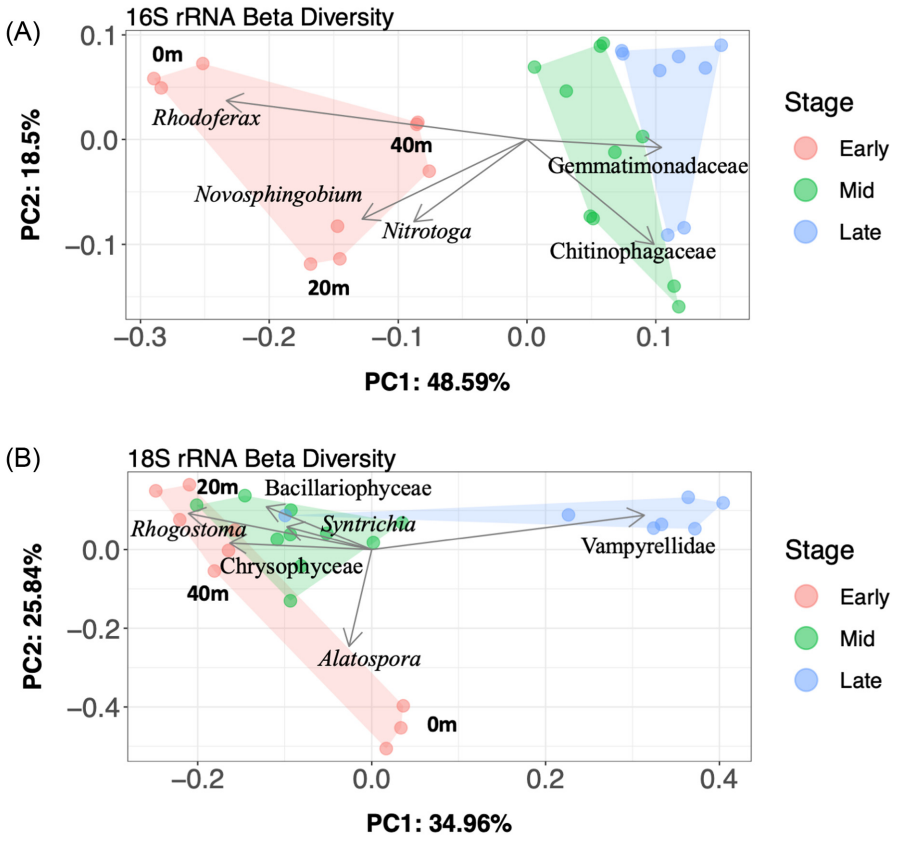

Figure 3. Principal coordinate analysis of UniFrac dissimilarities for bacterial and eukaryotic communities. Principal coordinate analysis (PCoA) ordination plot of bacterial **(A)** and eukaryotic **(B)** communities along the soil chronosequence. Samples are colored by chronosequence stage. Distances of samples within the "Early" stage are shown for clarity. Distance from the glacier terminus significantly affected both the bacterial (PERMANOVA $P = .001$; $R^2 = 0.48$) and the eukaryotic (PERMANOVA $P = .001$; $R^2 = 0.379$) communities. Analyses were based on ASV data matrices. Vector length is proportional to the correlation between the PCoA axes and the ASVs that contributed most to the observed dissimilarity among treatments (Similarity Percentage analysis, SIMPER).

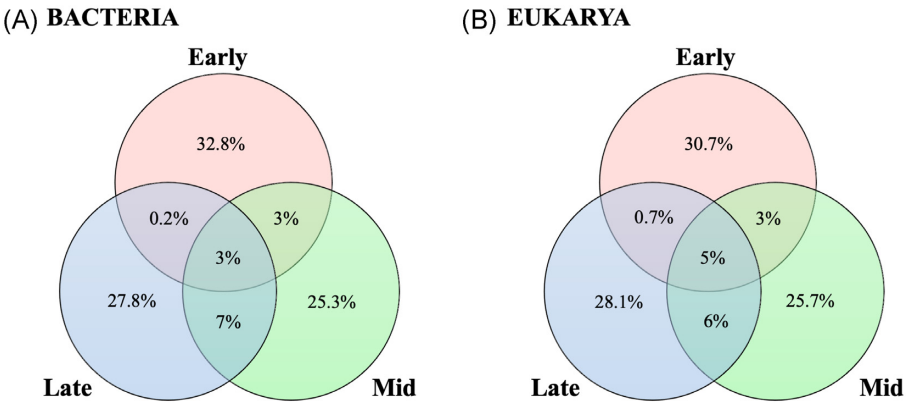

Figure 4. Shared ASVs among different chronosequence successional stages. Venn diagrams illustrating the % of bacterial **(A)** and eukaryotic **(B)** ASVs common to the three successional stages, between each pair of stages and specific to each stage.

Table 1. Compositional dissimilarities among bacterial, total eukaryotic, fungal, and algal communities along the soil chronosequence investigated. Analyses were done using ASVs matrices.

	Bacteria			Eukarya		
	Early	**Mid**	**Late**	**Early**	**Mid**	**Late**
*β*sor	0.789	0.796	0.748	0.814	0.8	0.759
*β*sim	0.696	0.74	0.703	0.779	0.765	0.695
*β*sne	0.093	0.055	0.045	0.035	0.035	0.063
	Fungi			**Algae**		
	Early	**Mid**	**Late**	**Early**	**Mid**	**Late**
*β*sor	0.782	0.797	0.786	0.793	0.769	0.686
*β*sim	0.705	0.754	0.712	0.759	0.711	0.588
*β*sne	0.077	0.042	0.074	0.033	0.058	0.098

*β*sor: Sørensen index of compositional dissimilarity.
*β*sim: Turnover component of compositional dissimilarity.
*β*sne: Nestedness component of compositional dissimilarity.

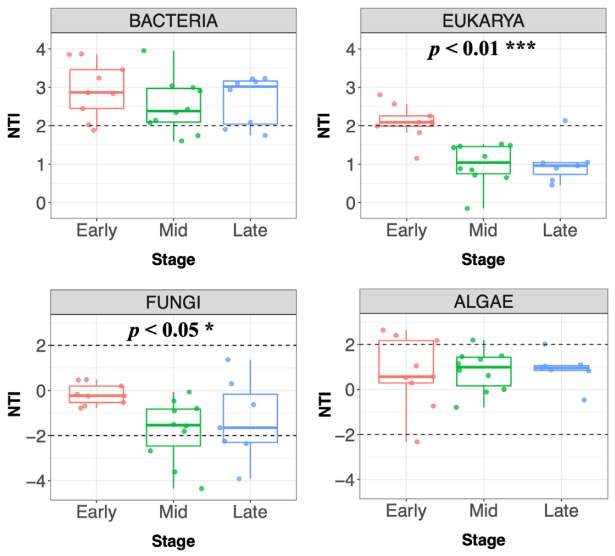

Figure 5. NTI patterns of different stages of the chronosequence. Box plots illustrating patterns of NTI in bacterial, eukaryotic, fungal, and algal communities at different stages of the chronosequence. Horizontal dashed lines present upper and lower significance thresholds at NTI = 2 and −2, respectively. NTI values are significantly different among the three chronosequence stages in the eukaryotic and fungal communities (ANOVA, $P < .05$) but not within the bacterial and algal communities. NTI values > 2 and < −2 indicate that deterministic processes are more important in shaping the community, while −2 < NTI < 2 values suggest that stochastic processes are more relevant.

sp. (Betaproteobacteria), a Chitinophagaceae sp. (Bacteroidetes), and a Gemmatimonadaceae sp. (Gemmatimonadetes).

The relative abundance of Acidobacteria, Gemmatimonadetes, and WPS-2 increased with distance from the glacier ($P < .05$), whereas Betaproteobacteria decreased with distance from the glacier ($P < .05$; Fig. 6A). In particular, Burkholderiales showed a rapid decrease. Sphingobacteriales (Bacteroidetes) exhibited a rapid increase up to 20 m from the glacier terminus but decreased significantly further along the chronosequence, and Aminicenantes and Flavobacteriales orders (Bacteroidetes) were almost exclusively found in soils at the glacier terminus. Chloroflexi within the Anaerolinae class were more abundant at the glacier terminus but were also present across the soil chronosequence. Taxonomy of closest matches for the most prevalent taxa is shown in Table S1a (Supporting Information).

Bacterial communities were significantly associated with deglaciation age ($P = .001$), pH ($P = .001$), and partially with TON ($P = .048$; Table 3). These factors collectively explained 44% of the bacterial community variation ($P < .05$; Fig. 7A and B).

Eukaryotic community

General eukaryotic diversity indicators increased significantly from "Early" to "Mid" sites and then declined significantly between "Mid" and "Late" sites (Fig. 2B), whereas eukaryotic evenness declined significantly from "Mid" to "Late" sites ($P < .05$), mainly because of the increased dominance of a Vampyrellidae (Cercozoa) ASV in the "Late" site soils. Fungal richness and diversity estimators were static across the chronosequence except for evenness, which was significantly higher in the "Early" and "Late" stages ($P < .05$; Figure S2a, Supporting Information). Algal richness and evenness did not significantly change along the chronosequence (Figure S2b, Supporting Information).

Eukaryotic community structure as a whole differed significantly among the three successional stages (PERMANOVA $P = .001$; $R^2 = 0.379$) (Fig. 3B). However, this was not the case for the fungal and algal communities: fungi showed partial overlapping for "Mid" and "Late" stages (Tukey's *post hoc* $P = .161$), while all stages overlapped in the algal community ($P = .453$, $R^2 = 0.075$; Figure S4a and b, Supporting Information). On the other hand, when analyzed by itself, the Cercozoa community was significantly different among the three main successional stages (PERMANOVA $P = .001$; $R^2 = 0.47$). Variation in eukaryotic communities within each successional stage was homogenous (betadisper $P > .05$). Like the Bacteria, eukaryotic hierarchical clustering anal-

Table 2. Bray–Curtis dissimilarity indices (Bc^{ij}) and turnover rates per year (expressed as percentage) of bacterial, total eukaryotic, fungal, and algal communities along the chronosequence. Bray–Curtis dissimilarity indices significantly decrease with increasing distance from the glacier for the bacterial community ($P = .034$) but not for the total eukaryotic, fungal, and algal communities ($P > .05$). Highest turnover rates per year occur between the closest samples to the glacier front (0–20 m) for the bacterial, eukaryotic, and algal communities. Turnover rates are expressed as percentages.

Succession stages (m)	Bray–Curtis dissimilarity indices (Bc^{ij})				Turnover rate per year			
	Bacteria	Eukarya	Fungi	Algae	Bacteria	Eukarya	Fungi	Algae
0–20	0.82	0.93	0.79	0.97	0.41	0.47	0.39	0.48
20–40	0.70	0.73	0.59	0.71	0.35	0.36	0.30	0.35
40–80	0.75	0.73	0.62	0.57	0.19	0.18	0.15	0.14
80–100	0.59	0.57	0.60	0.44	0.30	0.29	0.30	0.22
100–120	0.78	0.72	0.61	0.67	0.39	0.36	0.31	0.33
120–160	0.82	0.85	0.69	0.79	0.20	0.21	0.17	0.20
160–180	0.63	0.68	0.68	0.63	0.31	0.34	0.34	0.31
180–200	0.70	0.67	0.61	0.62	0.35	0.34	0.31	0.31
200–260	0.78	0.83	0.99	0.72	0.13	0.14	0.16	0.12
260–280	0.66	0.76	0.89	0.68	0.33	0.38	0.45	0.34
280–300	0.46	0.54	0.69	0.45	0.23	0.27	0.35	0.23

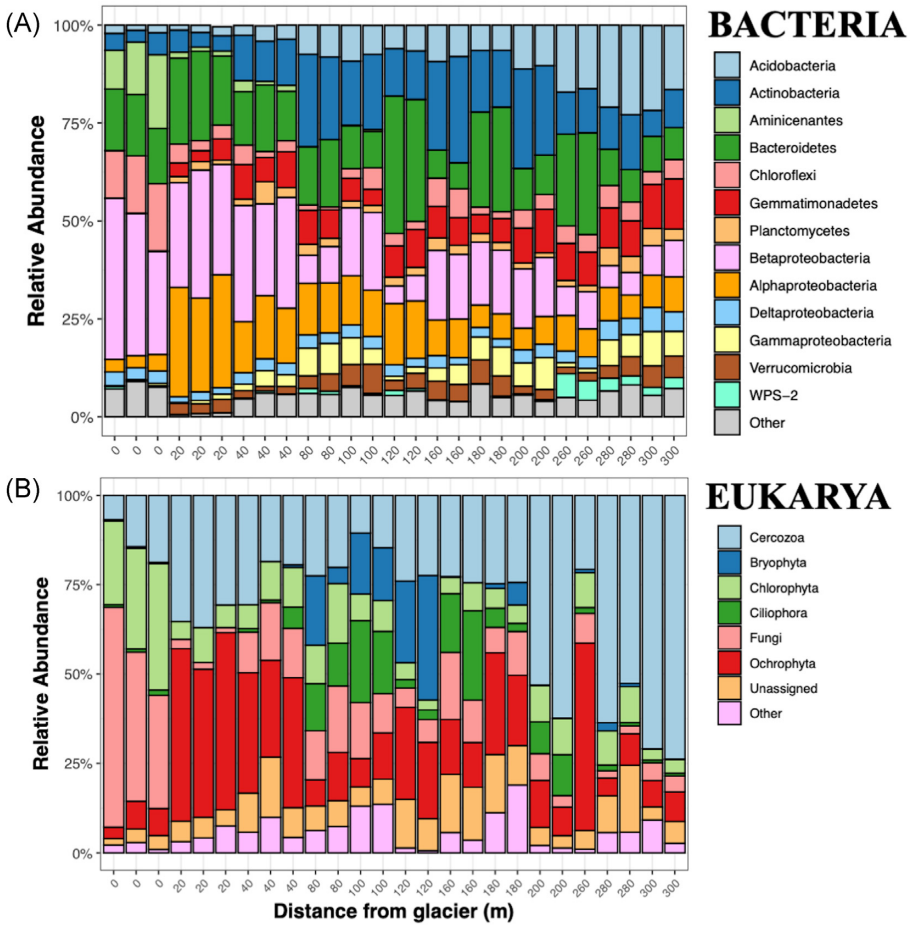

Figure 6. Relative abundance of bacterial and eukaryotic phyla along the chronosequence. Stacked bar graphs show relative abundance of dominant bacterial **(A)** and eukaryotic **(B)** phyla at increasing distances from the glacier terminus. Each bar represents a sample. Taxonomy is from the Silva 132 NR99 reference database. (A) The bacterial phylum Proteobacteria was subdivided into its four most abundant classes in the dataset. Acidobacteria, Gemmatimonadetes, and WPS-2 significantly increase with increasing distance from the glacier terminus ($P < .05$), while the Proteobacteria phylum significantly decreases ($P < .05$). (B) The eukaryotic phylum Cercozoa significantly increases with distance from the glacier terminus ($P < .05$).

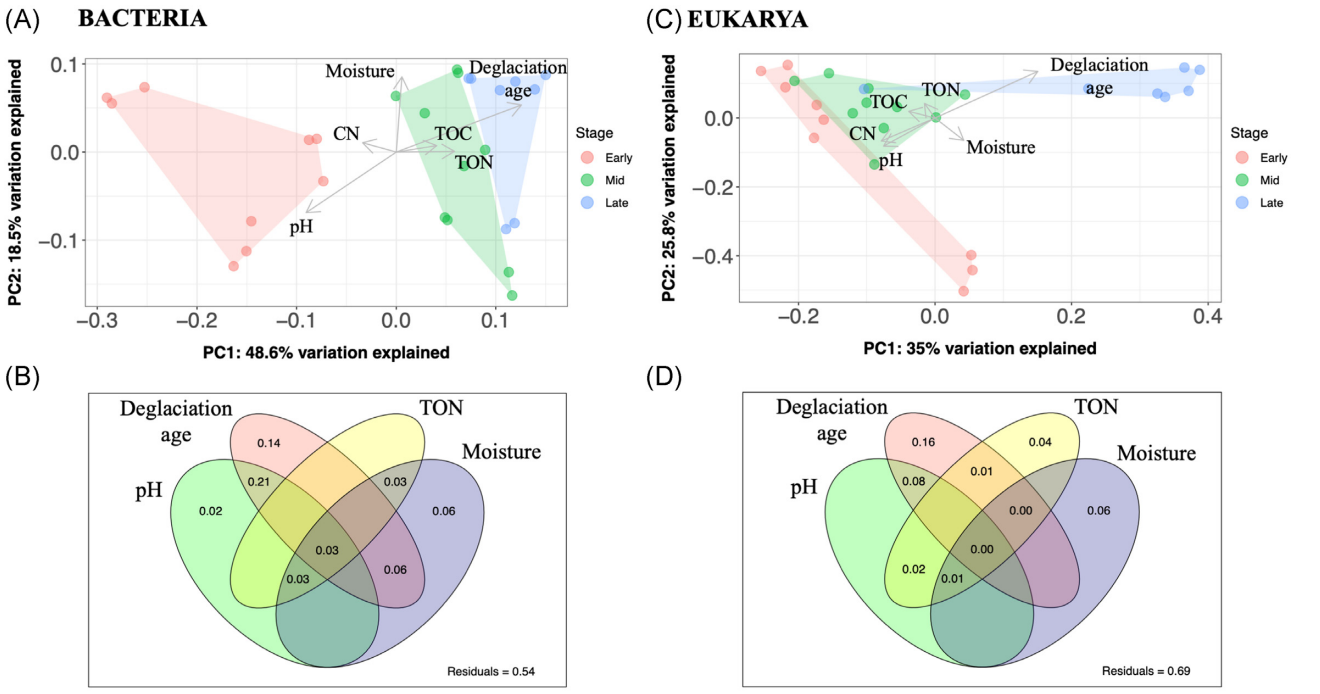

Figure 7. Environmental variables driving assembly of bacterial and eukaryotic communities. PCoA plots of UniFrac values for Bacteria **(A)** and Eukarya **(C)** with environmental variable correlations layered over community distances. Samples are colored by chronosequence stage. Vectors of environmental variables were overlaid onto the ordination space using the "envfit" function. The arrows indicate the direction of most rapid change in the variable and its length is proportional to the correlation between the ordination and the variable. Deglaciation age and pH are major drivers of the bacterial and eukaryotic community ($P < .05$). Individual and combined contribution of environmental variables to the succession of bacterial **(B)** and eukaryotic **(D)** communities are based on variation partitioning analysis. Deglaciation age explains the most variation in both bacterial and eukaryote communities (13% and 18%, respectively).

Table 3. Individual contribution of each environmental factor to the succession of bacterial and eukaryotic communities along the chronosequence based on variation partitioning analysis.

Environmental factor	Bacteria		Eukarya	
	Pseudo-F	P-value	Pseudo-F	P-value
Deglaciation age	13.98	0.001***	7.71	0.001***
pH	7.18	0.001***	2.64	0.026*
Moisture	1.84	0.117	1.96	0.08
Total organic carbon (TOC)	1.73	0.124	1.96	0.068
Total organic nitrogen (TON)	2.57	0.048*	1.98	0.083
TOC/TON ratio (C/N)	1.1	0.311	2.11	0.063

ysis showed that the earliest samples (0 m) cluster on their own (Fig. S1b, Supporting Information), yet the other clusters were not in accordance with chronosequence stages. Eukaryotic "Mid" and "Late" successional communities were more similar to one another (mean Bray–Curtis dissimilarity 0.748) than to "Early" successional communities (mean Bray–Curtis dissimilarity 0.849 and 0.855, respectively). This pattern applies to the fungal and algal communities as well. The degree of variation in ASV eukaryotic composition along the chronosequence showed slightly decreasing β_{SOR} values (Table 1). The turnover (β_{SIM}) component was comparable in the three stages of the chronosequence for Eukarya and fungi alone, whereas nestedness (β_{SNE}) was highest in the late stage for Eukarya and algae alone (Table 1). Overall, 84.7% of all ASVs were stage-specific, with the "Early" stage having a higher number of stage-specific ASVs, as seen with Bacteria (Fig. 4B). This number was lower for stage-specific fungal and algal ASVs (56.29% and 52.42%, respectively). Turnover rates according to the Bray–Curtis dissimilarity index were not affected by distance from the glacier ($P = .3342$) and the highest turnover rate per year occurred between the samples closest to the glacier front (0–20 m; Table 2). NTI values were > 2 only for the "Early" stage, but < 2 for "Mid" and "Late" stages (Fig. 5), suggesting that deterministic processes shape early eukaryotic succession while stochastic processes are more important in structuring the community in intermediate and later stages.

SIMPER analysis for the 18S rRNA data revealed that six ASVs comprising 27.2% of the total relative abundance accounted for most of the community compositional differences among chronosequence stages (Fig. 3B). The top six ASVs that explained the most variance between each stage pair were identified as *Alatospora* sp. (Ascomycota), a Crysophyceae sp. (Ochrophyta), *Rhogostoma* sp. (Cercozoa), *Syntrichia* sp. (Bryophyta), a Bacillariophyceae sp. (Bacillariophyta), and a Vampyrellidae sp. (Cercozoa).

Correlation between relative abundance of eukaryotic taxa with successional time showed that Cercozoa within the Vampyrellidae family increased significantly over successional time ($P < .05$) and dominated the community in the latest stages (Fig. 6B). Cercozoa ASVs of the earliest stages of the chronosequence (0 m) were uniquely found at this distance and were absent from all other distances (Figure S5, Supporting Information). At intermediate distances from the glacier (80–120 m) complex photosynthesizers within the Bryophyta (Pottiales order) bloomed in terms of relative abundance and then declined. Among Fungi, *Mrakia* and *Lecophagus* genera were almost exclusively found at the glacier terminus. No chytrids were found in the samples closest

to the glacier front, whereas members of the Cryptomycota were only found towards the "Mid" and "Late" stages of the chronosequence. The algal community did not show a distinctive beta diversity pattern; nonetheless, a few ASVs were nonrandomly distributed. A *Chloromonas* sp. ASV was exclusively found at the glacier forefront (0 m). Taxonomy of closest matches for the most prevalent taxa is shown in Table S1b (Supporting Information).

The eukaryotic community was significantly associated with deglaciation age ($P = .001$) and pH ($P = .026$; Table 3), which collectively explained 28% of the community variation ($P < .05$; Fig. 7C and D).

Network analysis

Both bacterial and eukaryotic ASV co-occurrence networks showed differences across the soil developmental stages (Fig. 8; Table 4). The number of interactions (edges, including both positive and negative ones) and node connectivity (average degree) were highest for both in "Early" soils and were lowest in "Mid" soils. This indicates high turnover of ASVs serving as connections. Bacterial communities formed a more complex and connected network, while the eukaryotic communities formed a more clustered network (Fig. 8A and C; Table 4). The modularity index for each stage of the chronosequence for Bacteria was < 0.4 suggesting that the network does not have a modular structure (Newman 2006). On the other hand, eukaryotic networks had a modularity value > 0.4 for both "Early" and "Mid" stages of chronosequence suggesting the existence of clusters of tightly connected taxa. The number of positive correlations was higher than the number of negative ones for each stage in both Bacteria and Eukarya.

Overall, the soil bacterial network was comprised of highly connected ASVs (> 5 edges per node) and about two-thirds of correlations were positive for Bacteria for each successional stage. Keystone taxa (largest number of degrees and greatest betweenness) at the "Early" soil stage mainly belonged to Proteobacteria, Bacteroidetes, and Aminicenantes (Fig. 8B), suggesting an important ecological role of these phyla in the assembly of bacterial communities in the earliest soils exposed after glacial retreat. Proteobacteria remained important players in shaping the topology of the bacterial network in "Mid" and "Late" stage soils, but to a lesser extent and with different taxa within the phylum.

Within Eukarya, the high turnover of ASVs serving as connections in the community did not affect the overall network topology. This suggests ecological vicariance of ASVs that serve as connection nodes. Highly connected nodes along the chronosequence mainly belonged to Fungi, Chlorophyta, Bacillariophyta, Ciliophora, and Cercozoa (Fig. 8C and D). The co-occurrence network of fungi showed that most connected nodes along the chronosequence were of unassigned taxonomy, while for algae, most connected nodes of "Early" soils were within Bacillaryophyta (Figure S6, Supporting Information).

Soil attributes and microbial communities

The chronosequence displayed a significant decrease in pH with time since deglaciation, while all other environmental variables measured did not display clear patterns (Table S2, Supporting Information). However, both TOC and TON had highest concentrations in the middle stage of the chronosequence.

The fitting of vectors of environmental variables onto the ordination space showed that deglaciation age was the major factor driving both the bacterial and eukaryotic community composition ($P = .001$, $F = 13.98$; $P = .001$, $F = 7.71$, respectively), but compositional variations were also shaped by other environmental variables (Fig. 7 and Table 3). The bacterial community was further explained by pH ($P = .001$, $F = 7.18$) and TON ($P = .048$, $F = 2.57$), while eukaryotic community was further explained only by pH ($P = .026$, $F = 2.64$). Deglaciation age was the single best predictor variable that explained both bacterial and eukaryotic community variation (13% and 18%, respectively). Variation partitioning analysis showed that a greater percentage of variation was unexplained in eukaryotes (67%) compared to bacteria (51%).

Microbial community production at different incubation temperatures

Microbial community production measured as ^{3}H-leucine incorporation significantly increased both with distance from the glacier front ($P < .001$, $F = 13.5$) and temperature ($P < .001$, $F = 15.14$). There was also a significant interaction ($P < .001$, $F = 4.8$) such that the magnitude of the positive effect of temperature depended on distance from glacier; temperature had the greatest positive effect on 100 m soils, and the smallest positive effect on 0 m soils (Fig. 9).

Nitrogen and carbon abundance and isotopic values

Both carbon and nitrogen abundance increased away from the glacier front to a maximum at about 100 m, then decreased further downfield (Table S2, Supporting Information). The C/N ratio remained consistent for most of the sequence, but C decreased more quickly than N at the end of the sampled sequence. Carbon isotope values were consistent throughout the profile, with a mean of $-25.25 \pm 0.33‰$. Nitrogen isotope ratios were lowest near the glacier, about 8‰, and increased to 10.5‰–11‰ further downfield.

Discussion

The most distinct community shift occurs within the earliest stages of succession

Our investigation showed primary succession patterns using a comprehensive analysis in a rapidly changing ecosystem: the Marr Ice Piedmont glacier near the Antarctic Palmer Station. The soil chronosequence spans a period of ∼30 years, which allowed a detailed description of the earliest phases of succession of newly exposed proglacial areas. A number of studies have now focused on succession patterns in glacier forelands of Bacteria, fungi, and algae, but to our knowledge no studies to date have simultaneously analyzed a full taxonomy of both Bacteria and Eukarya, as well as their network interactions.

The most dramatic shift in both bacterial and eukaryotic community structure was seen at the earliest successional stages, as revealed by the segregation of initial successional stages (Fig. 3A and B) and a high degree of turnover detected from the first few years after glacial retreat (Table 2). These initial abrupt changes suggest that the early colonizers are likely from the subglacial and/or supraglacial environment (Nemergut et al. 2007, Bradley et al. 2016, Rime et al. 2016, Khan et al. 2020), whereas later stages are likely due to colonization by more typical soil organisms from atmospheric deposition. This pattern was evident for both Bacteria and Eukarya. Future studies of glacial forefields should include a direct comparison of glacial ice, sediment, and atmospheric samples to establish the prevalence of the different sources of microbial colonizers in soil ecosystems after glacial retreat. In addition, we also revealed a decrease in dissimilarity of overall taxonomic composition between consecutive succession stages at increas-

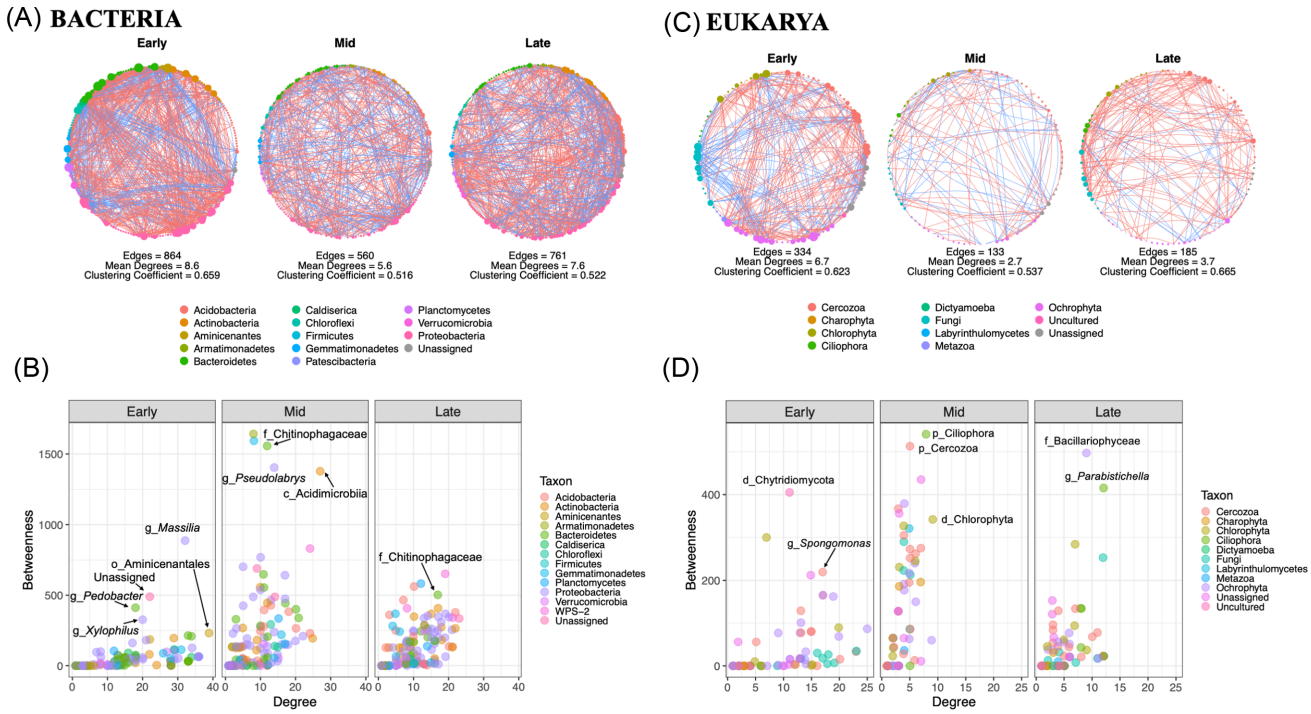

Figure 8. ASV co-occurrence networks based on correlation analysis across the successional gradient. Interaction among ASVs in the "Early" ($n = 9$), "Mid" ($n = 10$), and "Late" ($n = 8$) soils in bacterial **(A)** and eukaryotic **(C)** communities. A connection stands for a strong (Spearman's $\rho > |0.8|$) correlation. Red edges represent positive correlations while blue edges represent negative correlations. Each node represents an ASV. The size of each node is proportional to the number of connections (degree). **(B)** and **(D)** Betweenness centrality and degree of each ASV in the networks. Nodes with high betweenness centrality and high degree values are considered keystone taxa.

Table 4. Network statistics for microbial networks across the soil chronosequence. For bacterial and eukaryotic network diagrams see Fig. 8, for fungal and algal network diagrams see Figure S6 (Supporting Information).

Network metrics	Bacteria			Eukarya			Fungi			Algae		
	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late
Total number of edges[a]	864	560	761	334	133	185	32	18	45	152	40	46
Average clustering coefficient[b]	0.659	0.515	0.521	0.622	0.537	0.664	0.461	0.315	0.728	0.681	0.466	0.79
Modularity[c]	0.242	0.17	0.122	0.52	0.401	0.349	0.534	0.671	0.767	0.264	0	0.511
Average path length[d]	2.4	4.16	3.29	2.77	4.96	3.74	2.06	2.2	2.19	2.75	2.37	3.69
Average degree[e]	8.6	5.6	7.61	6.68	2.66	3.7	1.28	0.72	1.8	5.06	1.33	1.533
Number of positive correlations[f]	560	335	495	221	75	158	20	16	41	102	20	39
Number of negative correlations[g]	304	225	266	113	58	27	12	2	4	50	20	7

[a]Number of correlations between nodes.
[b]The degree to which nodes cluster together.
[c]A modularity value > 0.4 indicate the presence of nodes in the network that are more tightly connected between each other than with the rest of the network (Newman 2006).
[d]Average distance between all pair of nodes in the network.
[e]Average number of connections each node has to another node in the network.
[f]Correlation magnitude > 0.8
[g]Correlation magnitude < 0.8.

ing distances from the glacier front for both Bacteria and Eukarya (Table 2), which is expected due to selection of suitable taxa under more homogeneous environmental filters (Dini-Andreote et al. 2015). Early stages of the chronosequence were the most compositionally dissimilar, which may result from greater environmental heterogeneity and stochastic microbial influx from different sources, producing many niches with temporal instability (Dini-Andreote et al. 2015).

Alpha diversity shows an initial increase and then plateaus

Bacteria showed a significant increase in alpha diversity and then a plateau (Fig. 2A). Alpha diversity is expected to increase from early to later stages of succession due to a higher number of potential niches, habitat heterogeneity, and resource diversity (Jackson 2003, Fierer et al. 2010), especially if plants are

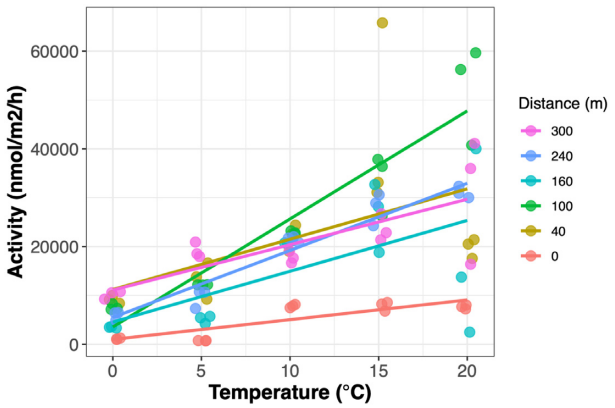

Figure 9. Microbial community response to different incubation temperatures. Microbial activity measured as ^{3}H-leucine incorporation in soils at increasing distances from the glacier terminus and incubated at increasing temperatures. Three assays were performed for each distance/temperature. Distance from the glacier front ($P < .01$, $F = 5.4$) and temperature ($P < .01$, $F = 15.14$) significantly affect microbial activity.

present and increase in density and richness (Porazinska et al. 2018). However, this was not the case for the eukaryotic community along the whole chronosequence (Fig. 2B). Alpha diversity values were highest in the middle of the chronosequence, which has been similarly shown in other deglaciated soils (Nemergut et al. 2007, Sun et al. 2016). Highest values of bacterial and eukaryotic diversity in mid-aged sites suggest there are more available niches for microbial communities compared with other stages, or that as the community develops, competition among taxa and/or predation become more important, thereby excluding some taxa.

Beta diversity patterns of bacteria and Cercozoa but not fungi or algae are affected by distance from the glacier

Analysis of beta diversity showed that community structure differed among the main three successional stages for both Bacteria and Eukarya (Fig. 3A and B). Variations in phylogenetic beta diversity were greater at initial stages of soil development, possibly as a result of the great dynamism imposed by the shift in environmental conditions. Interestingly, when the beta diversity within the eukaryotic community was parsed out for separate taxonomic groups, results showed that the structure of the Cercozoa (Rhizaria) phylum significantly differed among successional stages (Figure S5, Supporting Information), while fungi and algae did not. Fungi only showed a distinct cluster for the "Early" stage while the algal community was not affected by distance from the glacier front. Limitations to fungal and algal dispersal in terrestrial habitats are indeed relevant in other Antarctic sites (Archer et al. 2019). Overall, our study shows that fungal and algal communities have a more stochastic distribution compared to bacterial communities and the eukaryotic Cercozoa group along the chronosequence. To our knowledge, this is the first study showing clear spatial patterns for Cercozoa along a glacier chronosequence. Recent studies have highlighted that environmental selection structures cercozoan communities both spatially and seasonally (Fiore-Donno et al. 2019), but the importance of protistan taxa in proglacial soils remains mostly unknown.

Spatial scale affects the relative role of determinism versus stochasticity in eukaryotic communities but not in bacterial communities

An ongoing debate in ecology and microbial biogeography concerns the balance between the relative role of determinism versus stochasticity in structuring communities (Vellend et al. 2014), and more specifically, microbial successional patterns. In order to infer the relative importance of determinism versus stochasticity in bacterial and eukaryotic community assembly we measured NTI values of the three soil chronosequence stages. Deterministic processes mainly involve selection driven by environmental variables and biotic interactions (Zhou and Ning 2017), while stochastic processes are mostly driven by drift and/or historical contingencies (Nemergut et al. 2013). It is expected that initial community establishment is primarily dominated by stochasticity (Dini-Andreote et al. 2015); however, this was not the case for the bacterial community in our study (Fig. 5) indicating that there were strong selective forces acting on the bacterial community across the chronosequence. This finding is also supported by strong clustering of taxa in that specific bacteria tended to co-occur with closely related bacteria more than expected by chance (Horner-Devine and Bohannan 2006).

In contrast, NTI values for the eukaryotic community were on average > 2 only for the "Early" stage of the chronosequence, but < 2 for "Mid" and "Late" stages (Fig. 5) showing that deterministic and stochastic processes can play different roles during different successional stages (Powell et al. 2015, Jonsson et al. 2016). The finding of stronger deterministic processes in the "Early" stage is contrary to expectations and points to our general ignorance about the assembly of eukaryotic communities following glacier retreat and indicates that understudied factors like ecophysiology (Tian et al. 2017) and dispersal limitations (Schmidt et al. 2014) need much more study in early successional eukaryotes.

Taxonomy of the bacterial microbial community

As suggested by other studies (Rime et al. 2016, Yoshitake et al. 2018), the glacier probably represents the main environmental source seeding the earliest soils in deglaciated landscapes. Members of the Burkholderiales (Betaproteobacteria) are abundant in our early samples and significantly decline with distance from the glacier front. They are known glacier dwelling microorganisms (Philippot et al. 2011), which tend to dominate newly deglaciated soils in glacier forefields (Nemergut et al. 2007, Liu et al. 2016) and decline with soil age (Zumsteg et al. 2012, Jangid et al. 2013, Mateos-Rivera et al. 2016) as organisms better adapted to the soil environment invade. Members of this group, such as *Polaromonas* sp., can be nitrogen fixers and a number of studies have demonstrated they possess *Nif* genes (Darcy et al. 2011, Nash et al. 2018).

Cyanobacteria showed low relative abundance in all soils of the chronosequence, in agreement with results from other glacier forefield soils in maritime Antarctica (Strauss et al. 2012, Ji et al. 2016, Garrido-Benavent et al. 2020) and some other deglaciating systems (Sattin et al. 2009, Khan et al. 2020), but in disagreement with results from rapidly deglaciating soils in drier areas such as the Andes of Peru (Nemergut et al. 2007, Schmidt et al. 2008). However, the present study showed the presence of other potential phototrophic bacteria, such as *Rhodoferax* sp. (Betaproteobacteria), which can grow photoheterotrophically and photoautotrophically (Madigan et al. 2000) and, therefore, may contribute to primary production at this site. Chloroflexi were most abundant at the glacier forefront where they may have a crucial ecological role in contributing to C fixation (Lacap et al. 2011, Bryant et

al. 2012, Klatt et al. 2013). Members of the Aminicenantes phylum were found almost exclusively in the earliest samples of the chronosequence. This phylum has been found in higher abundance in anoxic and low temperature habitats (Farag et al. 2014), which can explain its presence restricted to the closest soils to the glacier. Members of the putatively nitrogen fixing Burkholderiales have higher abundance early in the chronosequence, consistent with lower δ^{15}N values, and may be particularly relevant since cyanobacteria are not found. Typical soil bacteria like Acidobacteria significantly increase along the chronosequence and are seen as indicators of soil development following glacial retreat (Nemergut et al. 2007, Bradley et al. 2016). Members of the Chitinophagaceae family (Bacteroidetes) also increase in abundance in later stages. Chitinophagaceae can degrade complex polymers such as chitin, a component of fungi and arthropods, frequently found in recently deglaciated soils (Zumsteg et al. 2012). The absence of an abundant nitrogen-fixing bacterial community within this chronosequence may indicate that microbial dwellers of this site mostly rely on both autochthonous carbon and nitrogen sources and depositions from the atmosphere.

Taxonomy of the eukaryotic microbial community

During the early stages of soil development, fungi are key components of the ecosystem, as they are able to mobilize nutrients (e.g. via rock bioweathering) facilitating their use by other organisms (Gorbushina and Broughton 2009). The fungal community shifted phylogenetically along our soil chronosequence, in contrast to other studies showing a relatively stable composition (Brown and Jumpponen 2014). This shift was more pronounced early in the chronosequence, while in the "Mid" and "Late" stages fungal clusters were partially overlapping. Fungal taxa were significantly more abundant in the "Early" stage soils of the chronosequence relative to the following stages. The uneven distribution of fungi along the chronosequence does not support the idea that most fungi present in recently deglaciated soils are dormant, as suggested by Jumpponen (2003), as we would expect a more stochastic distribution. Additionally, the "Early" stage hosted some fungal taxa that are typical for ice (*Mrakia* and *Lecophagus* genera), indicating that melting glacial ice is an important inoculum source for young soils (Dresch et al. 2019). The potential availability of a niche with autochthonous carbon and nitrogen sources could also explain why fungal abundance is highest closest to the glacier tongue. Previous studies have reported contrasting results on fungal diversity in deglaciated unvegetated areas (Bradley et al. 2014, Brown and Jumpponen 2014, 2015, Cutler et al. 2014, Hu et al. 2021); these inconsistencies suggest that unvegetated ecosystems emerging from glacial retreat worldwide are heterogeneous and host different fungal assemblages.

Algal community beta diversity did not significantly change along the chronosequence; however, there were obvious patterns of a succession in relative abundances of major photosynthetic groups along the chronosequence (Fig. 6B; Figure S3b, Supporting Information). Chlorophyta were the most highly abundant group in the most recently exposed soils and a highly abundant *Chloromonas* ASV (ASV_35) was only found in the earliest soil sample consistent with a glacial ice origin of this ASV (Vimercati et al. 2019b). The next closest soils to the glacier (20 and 40 m) showed a proliferation of diverse members of the Ochrophyta (including diatoms, and Chrysophyceae (Figure S3b, Supporting Information), which likely constitute the first allochthonous photosynthetic colonists. At intermediate distances from the glacier (80–120 m) more complex photosynthesizers within the Bryophyta (Pottiales order) bloomed in terms of relative abundance (Fig. 6B) and then declined as a parasitic Vampyrellidae ASV becomes the dominant ASV (Figure S5, Supporting Information). This successional pattern in algae and mosses also corresponds with the biogeochemical data in that there is a buildup of TOC and TON, (Table S2, Supporting Information) up through the Bryophyta stage of succession, followed by a decline in TOC and TON as the Vampyrellidae rose to ascendance late in the chronosequence. There is also a small but significant increase in δ^{13}C and δ^{15}N during the bloom of Bryophyta in the middle of the chronosequence (80–120 m), further indicating that there are trophic shifts occurring along the chronosequence.

Given the high diversity and dynamic succession of photosynthetic eukaryotes, and the near absence of cyanobacteria along the chronosequence, it is likely that eukaryotes are the dominant primary producers in this system as has been postulated for some other periglacial systems (Kastovská et al. 2005), but not others (Nemergut et al. 2007, Knelman et al. 2021). The early proliferation of eukaryotes in the present study may also indicate that the soils along this chronosequence are not nitrogen limited, which may give a competitive advantage to algae over N-fixing cyanobacteria. The δ^{15}N data also support this idea in that the values are quite high indicating high levels of trophic transfer of N and/or high levels of denitrification are occurring all along the chronosequence (Brankatschk et al. 2011, Ansari et al. 2013, Mohn et al. 2013, Schulz et al. 2013).

As indicated above, the phylum that increased the most in relative abundance along the soil chronosequence was Cercozoa, especially members of the Vampyrellidae family. Vampyrellidae are a group of naked filose amoebae generally known from soil and aquatic habitats where they are predators of algae, fungi, protozoa, and small metazoans (Berney et al. 2013). This group has recently been reported from other periglacial environments and may play a dominant role in periglacial habitats (Khan et al. 2019, Vimercati et al. 2019a, Thompson et al. 2020). Surprisingly, the Vampyrellidae ASVs found in the earliest soils of the succession are different from those that become increasingly abundant in mid-late stages, indicating a different ecological role for these taxa. The most abundant Vampyrellidae ASV, which makes up the majority of the eukaryotic reads, is only distantly related to the known closest matches in NCBI (92% identity, KX771957), revealing high endemicity of this taxon. Conversely, the closest matches for Vampyrellidae ASVs in soils next to the glacier front vary in their % identity, with one of them being a 100% match with a Vampyrellidae ASV retrieved from the top of Mt. Kilimanjaro (KX772000). Overall, our study indicates that ASVs within the Vampyrellidae are important players regulating productivity and diversity during primary succession along this glacial chronosequence.

Network complexity is the highest at the early stage of succession

Co-occurrence patterns observed generally result from taxa sharing similar ecological niches, metabolic dependencies and symbioses (Barberán et al. 2012, Zelezniak et al. 2015). While previous studies have shown co-occurrence patterns in recently deglaciated soils within chronosequences that involved vegetated stages, our study represents the first that includes both Bacteria and Eukarya network analysis of only unvegetated soils following glacial retreat. Our results show that the number of edges and complexity of microbial networks was highest at the early stage

of chronosequence and was lowest in the middle (for both Bacteria and Eukarya). The same pattern has been observed before in a salt marsh chronosequence (Dini-Andreote et al. 2014), a high-elevation successional gradient (Farrer et al. 2019) and the foreland of a receding glacier in the high Arctic (Dong et al. 2022). In general, communities with tight co-occurrence patterns and high complexity are less stable and more susceptible to disturbance (Saavedra et al. 2011), which may reflect the abrupt changes in composition seen in the earliest stages of chronosequence that experience strong environmental changes during the transition from ice to soil. Strong environmental dynamics associated with a changing environment at initial succession can generate daily fluctuations in temperature, nutrient availability, and moisture, leading to a temporally driven niche partitioning, which may elicit high phylotype coexistence. The great dynamism in environmental conditions at initial stages of succession may promote the emergence of multiple niches over short periods of time (Dini-Andreote et al. 2014). Alternatively, the tight co-occurrence at early stages could be the result of complex communities coming from the glacier terminus, as recent research has shown that chronosequences can exist on supraglacial environments (Darcy et al. 2017).

Co-occurrence networks can be compartmentalized into modules, within which nodes form closely associated subclusters and are expected to share environmental preferences. We observed modularity of Eukarya in "Early" and "Mid" stages (Table 4) and a lack of modularity in Bacteria. Within the eukaryotic community, fungi had even higher modularity values for each chronosequence stage, which is not surprising since fungi have been shown to display highly modular structures (de Vries et al. 2018, Ren and Gao 2019).

Overall, our analysis showed that bacterial communities had a more compact network topology during assembly, thus supporting determinism, whereas Eukarya exhibited less connected topologies suggesting their communities were more determined by stochastic processes. The limited functional information about many microbial taxa makes the interpretation of network patterns across succession difficult. More comprehensive analyses of functional traits will be key to clarify mechanisms of microbial turnover and networks along successions in glacier forelands.

Deglaciation age and pH are key drivers of community variation

A number of environmental variables along our soil chronosequence drove shifts in community phylogeny. The environmental factors analyzed collectively explained more variation of the bacterial community than that of eukaryotes, which along with the NTI values and network analysis, indicates that bacterial succession is more deterministic. Time since glacier retreat was the single variable that explained the most variation in both bacterial and eukaryotic community composition in this deglaciated foreland (Table 3), as seen in other similar environments (Nemergut et al. 2007, Kim et al. 2017). A decrease in pH generally occurs in soils exposed following glacier retreat due to weathering processes and plant root activity (Matthews 1992). Our chronosequence showed a significant decrease in pH (Table S2, Supporting Information), which proved to be a significant factor driving the taxonomic assemblage of both bacterial and eukaryotic communities (Table 3). Soil pH represents a key regulator in shaping patterns of bacterial communities in soils at regional scales (Fierer and Jackson 2006). Our investigation adds to a growing body of evidence showing that pH variation along proglacial chronosequences drives bacterial assemblages (Zumsteg et al. 2012, Bajerski and Wagner 2013, Kim et al. 2017), and it also shows how it is a significant factor shaping microeukaryotic communities. Moisture did not significantly affect the microbial community succession for either Bacteria or Eukarya. Water content has been considered an important factor driving microbial abundance and composition in polar soils (Niederberger et al. 2015), but it is not a major driver of bacterial community structure at our site. TOC did not play a significant role in the assembly of bacterial and eukaryotic communities, while TON marginally affected just the bacterial community. TOC and TON concentrations in our soil chronosequence are in the middle of the range reported for unvegetated glacier forefield soils (Bradley et al. 2014). Both TOC and TON peaked in the middle of the analyzed chronosequence. Carbon inputs can start via glacial runoff, aeolian deposition, or animal droppings (Bradley et al. 2014), as well as autochthonous ancient carbon reservoirs stored beneath the ice glacier (Bardgett et al. 2007). Nitrogen in our system could be supplied by nitrogen fixing colonizers, acquisition through allochthonous sources, and remineralization of ancient organic matter, similar to what is found in other glacier forefields (Brankatschk et al. 2011). Other potentially important variables that were not measured in this study and could be considered in future work include salinity, soil structure, dust deposition, and washout events (Dragone et al. 2021, Curtosi et al. 2007, Li et al. 2008).

Overall, the absence of a linear increasing trend in concentrations of TOC and TOC suggests that autochthonous/allochthonous inputs do not lead to appreciable soil C and N accumulation during the first 30 years after glacial recession. However, it is still unclear to what extent microbial life is responsible for initial build-up of carbon and nitrogen compared to external sources (Bradley et al. 2014). Lower δ^{15}N values near the glacier front are consistent with more N-fixation as a source of N, while more enriched values further downfield indicate allochthonous deposition, followed by partial denitrification (Brankatschk et al. 2011, Ansari et al. 2013). Other unmeasured environmental factors such as phosphorus, microbial characteristics and interactions, and interactions between microbes and viruses (Rastrojo and Alcamí 2018) can supplement changes along glacial forefields. In addition to being driven by abiotic parameters, it is thought that microbial succession could be partially autogenic and that the interwovenness of populations within a community can alter the abiotic characteristics of the surrounding environment (Walker and del Moral 2003).

Microbial community production depends on both temperature and distance from the glacier

Soils further from the glacier are progressively colonized by mesophilic microorganisms (Garcia-Lopez and Cid 2017) whose optimal temperature is higher than that of psychrophiles and psychrotrophs who are better adapted to the stable cold temperature of the glacier (Mateos-Rivera et al. 2016). We, therefore, hypothesized that a short-term increase in temperature would increasingly benefit microbial communities farther from the glacier front, resulting in higher activity. In accordance with our hypothesis, both temperature and distance significantly affected microbial production (which is a function of biomass and specific activity), and the magnitude of the growth response to temperature was greater in later successional stages compared to the earliest 0 m soils (Fig. 9). Communities closest to the glacier displayed lower activity at any incubation temperature tested than the rest of the chronosequence soils. Our results further support the notion

that the community that characterizes the earliest stages of the chronosequence is distinct from the rest of the chronosequence. The community at the earliest stages is primarily constituted by psychrotrophic microorganisms well-adapted to the glacier and subglacial environment with constant low temperature. It, therefore, makes sense that they are not as active at higher temperatures as those microorganisms that are found further from the glacier, as suggested in other studies (Mateos-Rivera et al. 2016). Future studies should focus on exploring how temperature increase influences the composition and diversity of the communities along the glacier forefield to parse out which members of the community are mostly involved in the response to shifts in temperature.

Conclusions

We examined the patterns and drivers of microbial succession along a chronosequence in a recently deglaciated area on the Antarctic Peninsula. The most dramatic compositional changes occurred in the earliest stages of succession, pointing to the existence of a critical window for the colonization of exposed soils following glacial retreat. Significant changes can occur in microbial community composition in polar latitudes within short temporal intervals and without the involvement of vascular plants, as suggested before for unvegetated soils from other polar and alpine receding glaciers. In parallel, network analysis showed the most complex topology at the earliest chronosequence stage, linking higher complexity with the high turnover observed. Compared with Bacteria and Cercozoa, fungal and algal communities were more structured by stochastic factors except for at the earliest stages of succession, providing evidence that drivers of community assembly are either distinct or operate at different spatial and temporal scales. Fungi and algae might be more dispersal-limited than bacteria, and therefore, more determined by historical contingencies. Deglaciation age and pH proved to be the most reliable environmental predictors of bacterial and eukaryotic communities. Our study adds to a growing body of knowledge on early successional patterns following glacial retreat, providing key information for a broader understanding of microbial turnover and predicting the potential ecological trajectories of soil microbial communities in response to climate change in polar systems. Although efforts have been made to predict the future distribution of microbial communities with mathematical models and more extensive investigations of these environments, we still lack the ability to globally predict future soil biodiversity and function in newly deglaciated landscapes (Chu et al. 2020). The growing body of metagenomic and metatranscriptomic work, along with taxonomic studies such as the one presented here, will aid in our understanding of how ecosystem function will change. Such work is critical given that deglaciation in alpine and polar environments will continue for the foreseeable future as Earth continues to undergo Anthropogenic climate change and the associated increases in temperature.

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Supplementary data

Supplementary data are available at *FEMSEC* online.

Conflict of interest statement. The authors declare no conflicts of interest regarding the publication of this paper.

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