Multiple-trophic patterns of primary succession following retreat of a high-elevation glacier
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Abstract. How multiple, interacting components of complex soil communities assemble within forefields of receding glaciers is still largely unknown, especially at high-elevation sites (>5000 m a.s.l.) where plant succession is very slow. To examine succession of soil communities across different trophic levels, we investigated four major soil groups: bacteria, fungi, nematodes, and other non-fungal non-nematode microbial eukaryotes at the Puca Glacier in the Peruvian Andes spanning 9-, 24-, and 89-year-old deglaciated soils. This is the first study of microbial communities, other than bacteria, at a high-elevation chronosequence in the Andes Mountains. In addition, we characterized soil biogeochemical properties (e.g., C, N, moisture, and pH) and rates of microbial enzyme activities associated with C, N, and P acquisition. We found significantly correlated increases in estimated richness and high species turnover in all soil groups along the chronosequence. These shifts in soil communities were significantly correlated with microbial enzyme activities and measures of C, N, moisture, and pH. Stoichiometric comparisons of enzyme activities showed phosphorus (P) and carbon (C) limitation of microbial activity across the entire chronosequence with no hint of nitrogen (N) limitation. Taken together, the observed shifts in soil communities and biogeochemistry indicate coordinated increases in trophic complexity and ecosystem functioning during the initial 90 yr of microbial succession along the post-glacial chronosequence of the Puca Glacier.

Key words: community complexity; glacial retreat; microbial communities; primary succession; trophic interactions.

INTRODUCTION

Records of natural glacial retreats are common and date back to the early 19th century. However, as humans exert more influence on climate warming, glaciers are receding at faster and faster rates (Marshall 2014). Although there have been many studies of the succession of bacterial communities following glacier retreat (reviewed in Nemergut et al. 2007, Bradley et al. 2014, Kim et al. 2017), how different components (e.g., microbial eukaryotes and microinvertebrates) of soil communities assemble within forefields of receding glaciers is still largely unstudied. For example, to our knowledge there have been no studies of trophic groups higher than bacteria at any receding glacier forefield in the entire Andes Range. Bacteria are generally better adapted to nutrient deficient environments than fungi and protists, because many bacteria can fix N2 and CO2 (via photoautotrophy and chemosynthesis), and therefore, bacteria should dominate early successional communities (Schaaf et al. 2011, Schmidt et al. 2014). Given the wider metabolic breadth of the bacteria as a whole, they should be less tied to the appearance of plants.
during primary succession. However, successional trajectories of these early communities may also be influenced by local and regional factors. For instance, bacterial communities in the forefield of the Lyman Glacier in the North Cascade Mountains were linked to the presence of plants more than fungal communities (Brown and Jumpponen 2014), but the opposite pattern was observed at Bläisen Glacier in Norway (Blaasilid et al. 2012). Likewise, fungal but not bacterial components of the soil community at the Lyman Glacier chronosequence responded to soil age (Brown and Jumpponen 2014), while the opposite was found in the forefields of lava flows of Mt. Hekla in Iceland (Cutler et al. 2014). A recent study at the forefront of Hailuogou Glacier in China suggested that differences can be attributed to a combination of many factors including site specificity and random effects (Jiang et al. 2018). We suggest that another factor playing a role in community assembly following glacial retreat could include biotic interactions with soil organisms at higher trophic levels.

The deglaciated forefield of the Puca Glacier in the Peruvian Andes has been characterized as having high abundances of cyanobacteria (e.g., Nostoc, Pseudanabaena, and Leptolyngbya) (Schmidt et al. 2008). In the early stages (spanning 0–80 yr) of the chronosequence, the diversity and evenness of bacterial communities were positively correlated with the age of soils (Nemergut et al. 2007) as were their activities such as rates of N-fixation (Schmidt et al. 2008). Later studies at this site established that microbial and plant colonization was limited by nutrients (mainly P; Castle et al. 2017, Darcy et al. 2018) and that soil nutrients were tied closely to microbial community succession (Knelman et al. 2014) suggesting that microbes and plants show similar successional patterns at this site.

Although previous studies at the Puca Glacier chronosequence provided insights into the succession of plant and bacterial communities, how communities of other soil microbiota such as fungi and microinvertebrates (e.g., nematodes) assemble along this post-glacial chronosequence is unknown. As many microinvertebrates feed on bacteria and fungi, the successional patterns of their communities could be tied to the patterns of their primary food sources. Nematodes are a major component of soil communities and are the most abundant and diverse microinvertebrates on Earth (Lambshead 2004, Hodda et al. 2009). Through their positioning as primary and intermediate consumers (Yeates et al. 1993, Bongers and Ferris 1999), they play important roles in soil ecosystem processes such as decomposition of organic matter, mineralization of nutrients (Schratzeberger et al. 2019), and plant productivity (Bernard et al. 2017). Hence, more detailed examination of nematode assemblages enables a better understanding of the complex interactions among members of the soil community.

To examine the process of succession of the entire soil community in this high-elevation glacial chronosequence, we collected replicated samples at three different spatial distances from the glacier representing 9-, 24-, and 89-year-old deglaciated soils. Our main objectives were to examine: (1) the diversity and composition of the main components of the entire soil community (e.g., bacteria, fungi, nematodes, and other non-fungal non-nematode microbial eukaryotes) along the chronosequence; (2) the potential functional roles of these groups along the chronosequence; and (3) the correlations among these soil groups and their relationship to edaphic factors. Given the relatively high pH of these soils which should be more conducive to bacterial than fungal life (Rousk et al. 2010, Zhang et al. 2016), and extremely low carbon and nitrogen content of these soils (Schmidt et al. 2008, Knelman et al. 2014), we hypothesized that succession of fungi, nematodes (especially fungivores and omnivores), and other eukaryotes would be slower than that of bacteria during the early stages of succession at this site.

**Methods**

**Study site and sampling**

The study was conducted at the forefield of Puca Glacier located in the Cordillera Vilcanota of the Peruvian Andes (13°46′24″ S, 71°04′17″ W, ~5000 m a.s.l.; Knelman et al. 2014). The site receives about 100 cm of annual precipitation mostly as snow (Nemergut et al. 2007) and soil temperatures can oscillate between −10°C and over 25°C on a daily basis during the dry season (Schmidt and Vimercati 2019). Soil samples were collected on 23 March 2015 from the same
transects that were previously described by Knelman et al. (2014), which also correspond to transects 2, 3, and 4 as described by Castle et al. (2017). Briefly, we sampled three transects representing advancing stages of succession having been exposed by the retreating Puca Glacier for approximately 9, 24, and 89 yr. The 9-year-old location was identified by direct field observations of multiple expeditions between 2005 and 2012, and the age of older locations was based on aerial and satellite photographs taken between 1931 and 2003 and ground truthing the locations based on landmarks on the ground as described in more detail in previous publications (Nemer-gut et al. 2007, Seimon et al. 2007, Schmidt et al. 2008, Knelman et al. 2014). In contrast to the presence of plants (25–50% plant cover) at the 89-year-old site, there were no plants in the youngest soils and only biological soil crusts and some mosses at the 24-year-old site (Knelman et al. 2014). At each successional stage, four composite samples (consisting of 30–50 g subsamples) from the top 5 cm of soil within ~400 cm² area were collected, placed into sterilized Ziplock bags, and gently homogenized. The four composite samples at each successional stage were at least 5 m from each other along the transect. Collection spoons were surface sterilized with ethanol wipes between samples to eliminate cross contamination. Samples were frozen overnight and shipped to the laboratory at the University of Colorado where there were immediately subsampled for specific biological and biochemical analyses as described below and subsequently processed or frozen till later.

DNA extraction and PCR

For the analysis of bacteria, fungi, and non-fungal non-nematode eukaryotic total DNA was extracted from ~0.3 g of soil subsamples using PowerSoil DNA Isolation kit according to the manufacturer’s instructions. Because 0.3 g of soil is not enough to accurately represent nematodes, nematodes were extracted from ~50 g of soil using a mobility-dependent method (Whitehead tray) over a period of 24 h (Porazinska et al. 2018) and counted to a trophic group level (Yeates et al. 1993) under an inverted microscope. Extracted nematodes were reduced to 0.5 mL, transferred to PowerSoil bead-beating tubes, and then processed for DNA as described above. Multiplexing metabarcoding primer sets were used to amplify bacterial 16S (515F/806R), eukaryotic 18S (1391F/EukBr), and fungal ITS1 (ITS1-F/ITS2) ribosomal regions according to the Earth Microbiome Project (http://www.earthmicrobiome.org/protocols-and-standards/; Amaral-Zettler et al. 2009, Bellemain et al. 2010, Caporaso et al. 2012). PCR was carried out in triplicates for each primer set and amplified samples were purified and normalized using Sequal-Prep Normalization Kit (Invitrogen, Carlsbad, California, USA), then pooled together to form three amplicon libraries (one for each marker gene) and sequenced on three Illumina MiSeq (Illumina, San Diego, California, USA) (2 × 300 bp) lanes at the University of Colorado BioFrontiers sequencing facility.

Soil biogeochemistry and microbial activity

The detailed methods were described elsewhere (Porazinska et al. 2018). Briefly, soil moisture (%) was measured by drying 5 g of soil for 48 h at 60°C. Dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and inorganic N (NH₄⁺, NO₃⁻) were measured using soil extracts (5 g soil in 25 mL 0.5 mol/L K₂SO₄). After mixing, soil slurries were centrifuged for 3 min at 20,124 × g and immediately filtered through 0.3 μm glass fiber filters. A Shimadzu total organic carbon analyzer was used to measure DOC and TDN, and inorganic N (IN) was quantified with Lachat QuikChem 8500 Flow Injection Analyzer (Lachat Instruments, Loveland, Colorado, USA) and Synergy 2 Multi-Detection Microplate Reader (BioTek Instruments, Winoski, Vermont, USA). Total N (%) was measured by combustion of ~50 mg air-dried and ground soil using a Thermo Finnigan Flash EA 1112 Series CHN analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Microbial biomass C and N (µg/g dry soil) were estimated by treating 5 g of soil with 2 mL alcohol-free chloroform. After 24 h of incubation, the soil slurry was vented for 1 h to release chloroform and then measured with K₂SO₄ extraction method as described above. Activities (nmol h⁻¹ g⁻¹ dry soil) of seven extracellular enzymes involved in N (leucine aminopeptidase—LAP and N-acetylglucosamine—NAG), C (α-glucosidase—AG, β-glucosidase—BG, β-xylanase—BXYL, cellobiosidase—CBH), and
P (phosphatase—PHOS) acquisition were measured in soil slurries made of 1 g of soil mixed together with 125 mL 50 mmol/L sodium bicarbonate buffer adjusted to pH 8.0 and homogenized at 3000 rpm for 1 min using Ultra-Turrax homogenizer. The sample slurries were transferred to 96-well plates with controls and substrates and incubated at 13°C for 22 h (Weintraub et al. 2007, King et al. 2010, Castle et al. 2017). The activity of enzymes was measured using Synergy HT Multi-Detection Microplate reader (BioTek, Winooski, Vermont, USA). Stoichiometric analyses using the seven enzymes were done to estimate which nutrients were most limiting to microbial activity at the time the soils were collected as discussed elsewhere (Hill et al. 2012, Schmidt et al. 2016, Bueno de Mesquita et al. 2018). To derive enzyme ratios (C/N, N/P, and C/P) indicative of which soil nutrients are most limiting to microbes (Sinsabaugh et al. 2009), mean value of the sum of enzymes associated with C (AG+BG+CHB+BXLY) and N (LAP+NAG) were used.

Sequencing data processing
QIIME 2 (Caporaso et al. 2010, Bolyen et al. 2019) was used to process all sequencing reads. First, the raw sequences were demultiplexed to each sample based on unique barcode sequence, and then, the forward and reverse sequences were joined to reduce sequence errors. The primer sequences of the 16S/18S/ITS region were further trimmed from the joined sequences. The average length of final reads for 16S and ITS was 250 and 130 bp for 18S. The dada2 (Callahan et al. 2016) pipeline was used to check chimeras and generate amplicon sequence variants (ASVs) tables at 100% similarity (Callahan et al. 2017). The taxonomy was assigned to the ASVs using blast (Camacho et al. 2009). The inhouse curated Silva_111 database (Pruesse et al. 2007) was used to assign taxonomy for bacteria and eukaryotes (including nematodes), while UNITE version8 (20190202) for fungi (Nilsson et al. 2019).

Community analyses
The ASV tables were filtered depending on the sample type and specific sequenced region. The archaea and mitochondrial ASVs were removed from the 16S ASV table, for the 18S non-fungal eukaryotic ASV table, fungi, nematode, and plant ASVs were removed and these are designated from now on as “other eukaryotes.” For nematode 18S ASV and fungal ITS ASV tables, only nematode and only fungal ASVs were retained. The unassigned ASVs were removed from all of the ASV tables prior to analyses. The ASV tables generated in QIIME 2 were transferred into R V.3.5.3 (R Development Core Team 2019) for all downstream analyses. The number of reads of bacterial, fungal, and non-fungal eukaryotic ASVs was standardized to per 1 g dry soil, and of nematode ASVs to per 50 g dry soil. Alpha diversity indices (Chao1 and Shannon) were calculated using phyloseq package (McMurdie and Holmes 2013) and beta diversity (based on Bray–Curtis distance dissimilarity matrices) was calculated using vegan (Oksanen et al. 2011).

Because successional community development may result in changes of species functional traits, we assigned putative functional profiles to our fungal ASVs using FUNGuild (Nguyen et al. 2016) and to nematode ASVs using the Outline for Soil Ecologists (Yeates et al. 1993).

Statistical analyses
Venn diagrams of the unique and standardized ASVs for each soil group were generated using R package eulerr (Larsson 2020). The presence of significant differences in alpha diversity indices (Chao1 and Shannon) among communities from soils of different successional stages was tested using ANOVA with age/distance along the chronosequence as the main factor followed by Fisher’s least significant difference (LSD). The presence of significant differences in community composition (beta diversity) was tested with PERMANOVA with 10,000 permutations in R package vegan (Oksanen et al. 2011). Because these tests were based on untransformed data (differences in abundance and diversity likely reflect real biology and ecology in this system), the tests were repeated using Aitchison distance, which has some advantages for compositional data (Aitchison et al. 2000). Details of the Aitchison distance and tests are included in the Appendix S1. To determine whether alpha diversity patterns were similar across all soil communities, we compared the correlation of richness estimator Chao1 among the different communities (e.g., bacteria vs. fungi, bacteria vs.
nematodes, etc.) using a model-II regression implemented in R package lmodel2 (Legendre and Oksanen 2018). Because we ran six comparisons, we adjusted the P values with the False Discovery Rate correction (Benjamini and Hochberg 1995) with the R package stats (R Development Core Team 2019). The abundance of most notable taxa (e.g., bacterial genera) and functional traits (e.g., fungal saprotrophs and bacterial-feeding nematodes) was tested with ANOVA as above. All values were considered significant at \( P < 0.05 \) unless otherwise stated.

Differences in soil chemistry along the chronosequence were tested with ANOVA as above. Relationship of soil communities with geochemical characteristics was tested using envfit function in R package vegan, and significant soil characteristics were reported via PCoA ordination plots at \( P < 0.05 \). GDM (Ferrier et al. 2007) was used to compare how bacterial, fungal, nematode, and other eukaryote communities responded to soil geochemical gradients. Predictor data for GDM were highly co-correlated and were clustered using \( k \)-means clustering of pairwise unsigned correlation distance \((1 - |r|)\), using \( k = 5 \) which was determined using the elbow method. Clusters were (1) PHOS, (2) inorganic N, (3) microbial N and microbial C, (4) DON, DOC, and LAP, and (5) pH, total organic N, total organic C, AG, BG, BXYL, CBH, NAG, and successional age. GDM was run with PHOS, inorganic N, microbial C, DOC, and age as predictor variables, explaining compositional dissimilarity (Aitchison distance using the isometric log ratio transformation; Egozcue et al. 2003) of community data for each of the four biological groupings mentioned above. We used GDM v1.3.11 (Manion et al. 2016).

To better understand potential roles of specific taxa at each successional stage, we used the multipatt function in the indicspecies package (De Caceres and Legendre 2009).

**RESULTS**

**Community diversity**

There was a general pattern of increasing alpha diversity along the chronosequence for all soil groups. The bacterial community ASV table amounted to a total of 4843 ASVs with 48,510 total sequences. The total number of observed ASVs increased with increasing age of soils with only 207 ASVs being shared among all three stages of the successional gradient (Appendix S1: Fig. S1A). Diversity measures (Chao1 and Shannon) showed that bacterial communities were significantly more diverse in the 89-year-old soils than in the younger soils, and the communities in the 9- and 24-year-old soils were not significantly different from one another (Table 1).

There were 463 soil fungal ASVs with 256,912 sequences. Both diversity measures (Chao1 and Shannon) of fungal ASVs significantly increased along the chronosequence (Table 1, Appendix S1: Fig. S1B).

The number of nematode ASVs was at least an order of magnitude lower than of all investigated soil groups with a total of 67 ASVs represented by 77,980 sequences. Only four ASVs were present in the 9-year-old soils, compared with 62 ASVs in 89-year-old soils, and only two nematode ASVs (both Aphelenchoidid fungal feeders) were shared among the three sites (Appendix S1: Fig. S1C). Both Chao1 and Shannon diversity measures showed that the most diverse nematode community was present in the 89-year-old soils (Table 1).

There were 941 other eukaryotic (non-fungal, non-nematode) ASVs with 219,106 sequences. Similar to the bacterial and nematode communities, the other eukaryotic community was characterized by the significantly higher richness in the 89-year-old soils (Table 1; Appendix S1: Fig. S1D). However, the Shannon diversity index was highest in the 24-year-old soils (Table 1).

The Chao1 richness estimates correlated significantly between all pairs of communities (Fig. 1). The correlation between alpha diversities of fungi and nematodes was particularly strong (Fig. 1D), and the relationship between bacteria and non-fungal, non-nematode eukaryotes richness was a close second (Fig. 1C).

**Community composition, structure, and function**

The community composition and structure (beta diversity) were significantly different among the three locations along the chronosequence for all four soil community groups (Table 2). Successional age along the chronosequence explained ∼40% of the variance of the soil communities with communities from the same location closely clustering together and clearly
separating from communities of other locations (Fig. 2A–D) and this pattern remained unchanged when examined using Aitchison distance (Appendix S1: Table S1, Fig. S2).

Proteobacteria, Bacteroidetes, and Acidobacteria were the most common and abundant phyla in all our samples (Fig. 3A). Interestingly, despite high soil pH (~8), the most abundant ASVs belonged to a presumptive photosynthetic Chloracidobacterium (Acidobacteria) distributed fairly uniformly across all successional stages. Among the other most abundant photosynthetic taxa were cyanobacteria (e.g., Microcoleus, Nostoc, and Phormidium), which unlike Chloracidobacterium, were generally more abundant in the youngest soils (Fig. 3A; Appendix S1: Table S2). The most

Table 1. Alpha diversity of soil communities at the Puca Glacier chronosequence.

| Year | Bacteria Chao1 | Bacteria Shannon | Fungi Chao1 | Fungi Shannon | Nematodes Chao1 | Nematodes Shannon | Other eukaryotes Chao1 | Other eukaryotes Shannon |
|------|----------------|-----------------|-------------|---------------|-----------------|-------------------|-----------------------|------------------------|
| 9    | 558 a          | 5.43 a          | 25.5 a      | 1.65 a        | 2 a             | 0.47 a            | 89 a                  | 3.17 a                 |
| 24   | 605 a          | 5.44 a          | 52 b        | 2.24 ab       | 5.75 a          | 1.05 a            | 105 a                 | 2.15 b                 |
| 89   | 966 b          | 6.22 b          | 96 c        | 3.07 b        | 26.25 b         | 2.17 b            | 225 b                 | 3.12 a                 |
| F    | 8.64           | 31.59           | 18.86       | 5.29          | 39.8            | 10.91             | 8.29                  | 3.91                   |
| P    | 0.008          | <0.0001         | <0.001      | 0.03          | <0.0001         | 0.007             | 0.01                  | 0.06                   |

Note: Different letters (a, b, c) indicate the presence of a statistically significant difference as indicated by the F statistic at \( N = 4 \) and \( P \) values measured by ANOVA.

![Fig. 1](https://www.esajournals.org/doi/10.1890/10010860/fig1.png)

Fig. 1. Pairwise model-II regressions of the Chao1 estimate of alpha diversity for: (A) bacteria and fungi, (B) bacteria and nematodes, (C) bacteria and other eukaryotes, (D) fungi and nematodes, (E) fungi and other eukaryotes, and (F) nematodes and other eukaryotes. Model-II correlation coefficient \( r \) and false discovery rate-corrected \( P \) values are displayed. Shaded regions are 95% confidence intervals.
abundant Proteobacteria in all soils were Sphingomonadales (e.g., Sphingomonas), but other Alphaproteobacteria, especially Rhizobiales (including some known to be associated with plants) increased with soil age (Appendix S1: Table S2). Likewise, the more abundant Burkholderiales (Betaproteobacteria) and Pseudomonadales (Gammaproteobacteria) (Appendix S1: Table S2) had their highest abundance in the 89-year-old soils. Verrucomicrobia, represented predominantly

Table 2. Beta diversity of soil communities at the Puca Glacier chronosequence.

| Organisms          | $F$  | $P$    |
|--------------------|------|--------|
| Bacteria           | 0.39 | <0.001 |
| Fungi              | 0.27 | 0.003  |
| Nematodes          | 0.32 | 0.04   |
| Other eukaryotes   | 0.36 | <0.001 |

Note: The presence of a significant difference was measured by the PERMANOVA test using Bray–Curtis dissimilarity distance matrix.

Fig. 2. Principal coordinate analyses for communities of: (A) bacteria, (B) fungi, (C) nematodes, and (D) other eukaryotes. Significant ($P < 0.05$) soil geochemical characteristics that correlated with each community were plotted using the function envfit. The arrow indicates positive/negative relationship, and stronger correlations are indicated by longer arrow lengths.
by *Chthoniobacter* (Fig. 3A; Appendix S1: Table S2), generally exhibited a similar pattern, however within *Chthoniobacter* many individual ASVs deviated from that general pattern (e.g., some ASVs were more abundant in the youngest soils).

The most abundant fungi along the successional gradient fell into three main supposed functional groups according to FUNGuild: (1) lichenized ascomycete fungi, (2) endophytic/saprotrophic fungi, and (3) fungi with unknown resource preference (Fig. 4A, Appendix S1: Table S2). Among lichenized fungi, ascomycete Verrucariaceae dominated (56.5% of all ASVs) and showed a non-significant trend of being more common in the youngest soils (Appendix S1: Table S2). In addition to lichenized fungi, endophytic/saprotrophic fungi from *Mortierella* (Mortierellomycota) were a substantial component of fungal communities particularly in the oldest soils (Fig. 3B; Appendix S1: Table S2), but the pattern was not significant. Taxa categorized via FUNGuild as plant pathogens (e.g., *Alternaria* and *Leptosphaeria*), and
symbiotic endomycorrhizal fungi (e.g., *Glomus*) were only present in the 89-year-old soils (Fig. 4A; Appendix S1: Table S2).

In the 9-year-old soils, only three fungal-feeding (*Aphelenchoides*) and one bacterial-feeding (*Tridentulus*) nematode ASVs were observed (Figs. 3C, 4B). In the 24-year-old soils, the nematode community was slightly more complex with a single occurrence of an omnivore (*Mesodorylaimus*), two bacterial-, and mostly fungal-feeding species (9 in total) being present. In contrast, the 89-year-old soils contained complex nematode communities with all known trophic groups represented (Fig. 4B). This diversity at the trophic level was mirrored by the species diversity (Fig. 3C). For example, predatory nematodes in the family Nygolaimidae and plant parasites in the family Anguinidae were only present in the oldest soils (Appendix S1: Table S2). Bacterial feeders in the oldest soils were dominated by *Plectus*, *Teratocephalus*, and *Prismatolaimus*. With the exception of *Plectus*, all bacterial-feeding taxa were present only in the oldest soils.

Within the other eukaryotic community, the abundance of metazoan ASVs (mostly rotifers and arachnids) were highest within soils of the intermediate age (Fig. 3B) but the pattern was not significant. Collembola, Tardigrada (*Hybisiidae*), and Turbellaria were virtually absent within the youngest soils (Appendix S1: Table S2). Interestingly, the pattern of nearly complete absence in younger soils was also common to all types of algae including golden (Chrysophyceae), green (Chlorophyceae and Ulvophyceae), and red (Rhodophyceae) algae (Appendix S1: Table S2).

In contrast to numerous bacterial indicator taxa for each of the soil age categories along the chronosequence representing all main phyla (Appendix S1: Table S2), only a handful of indicator taxa were found among fungi (e.g., *Mortierella* for 9-year-old soils, *Serendipita* and *Leohumicola* for 24-year-old soils, and *Mortierella*, *Tetracladium*, *Didymellaceae*, and *Verrucariaceae* for 89-year-old soils), nematodes (e.g., bacterial-feeding *Plectus* and *Rhabdolaimus* for 89-year-old soils), and other eukaryotes (e.g., *Spumella* for 9-year-old soils, bdelloid rotifer for 24-year-old soils, and green algae *Scenedesmus* and *Desmochloris* for 89-year-old soils; Appendix S1: Table S3).

Fig. 4. Number of sequences observed for: (A) fungal and (B) nematode functional groups (A). Abbreviations are Symbio, symbiotroph; FP, fungal pathogen PP, plant pathogen; Sapro, Saprotroph; Ectomyco, ectomycorrhizal; AP, animal pathogen; Endo-Sapro, endophyte-saprotroph.
Relationships with soil biogeochemistry

Nearly all biogeochemical measures (except for DON) were significantly different among sites along the chronosequence (Appendix S1: Table S4) and correlated with beta diversity of all soil groups (Fig. 2A–D). Unlike soil pH with its highest values (8.5) in the youngest soils, other soil measures were positively correlated with the age of soils (Fig. 2). While bacterial and fungal communities positively correlated with both C and N soil biochemical measures (Fig. 2A, B), the nematode and other eukaryotic communities were correlated only with N measures (Fig. 2C, D).

Community composition of bacteria, fungi, nematodes, and other eukaryotes was highly predictable using biogeochemical data based on GDM. GDM models explained 83.4% of community dissimilarity for nematodes, 83.7% for fungi, 84.7% for other eukaryotes, and 75.8% for bacteria (Fig. 5). The heights of GDM splines indicate their relative importance in models (Ferrier et al. 2007, Fitzpatrick and Keller 2015), meaning PHOS was the most important predictor of bacteria, and other eukaryotes when all other variables were accounted for in the model. PHOS was fairly important for fungi and nematodes as well. Microbial C was the strongest predictor of nematode and fungal community composition, although microbial C was strongly correlated with microbial N, so this result could relate to either. Successional age was not an important variable in models for nematodes, fungi, or other eukaryotes. This result does not necessarily mean that age is not correlated with eukaryote community composition, but instead that age does not explain any variability in community composition that is not explained by other variables in the model. Age, however, was marginally important for bacterial community composition.

Microbial biomass C and N and activity of microbial enzymes associated with C and N acquisition significantly increased along the chronosequence (Appendix S1: Table S4) whereas the activity of phosphatase did not change along the chronosequence. The mean activity of phosphatase (491 nmol·h⁻¹·g⁻¹ dry soil) was three times higher than that of C-associated enzymes (138 nmol·h⁻¹·g⁻¹ dry soil), and over an order of magnitude higher than that of N-associated enzymes (35 nmol·h⁻¹·g⁻¹ dry soil). This stoichiometric comparison by using the C:N and N:P ratios (Hill et al. 2012) is visualized in Appendix S1: Fig. S4 suggesting strong soil P deficiency and some C deficiency along the entire chronosequence.

Discussion

We investigated communities of major soil groups including bacteria, fungi, nematodes, and other non-fungal and non-nematode eukaryotes (e.g., protists, algae, and microarthropods) in the forefield of the Puca Glacier spanning 9- to 89-year-old soils. Previous work at this intensively studied site focused on bacteria and plants in relation to the succession of soil biogeochemical processes (Nemerbut et al. 2007, Schmidt et al. 2008, Knelman et al. 2014, Castle et al. 2016, 2017, Darcy et al. 2018). The study reported here builds on these earlier studies to add a more complete picture of the entire soil microbial community and its interactions with soil biogeochemistry along the chronosequence. As with previous studies of this chronosequence (Nemerbut et al. 2007, Schmidt et al. 2008, Knelman et al. 2014), this study shows the expected increases in bacterial diversity and biogeochemical functioning with increasing soil age. More importantly, the present study demonstrates for the first time that diversity of other soil microbial groups increased in parallel with the bacterial community (Table 1) and that these groups were similarly and predictably related to soil biogeochemical characteristics (Figs. 2, 5). This indicates that even in this extreme, high-elevation (5000 m a.s.l.) environment, all of the various components of the community increased in a more-or-less concerted fashion. While this may not be surprising for more temperate soils from lower elevations, it is somewhat surprising for an extreme environment where we previously hypothesized that bacteria would have an advantage due to daily temperature extremes, high soil pH, and the paucity of nutrients (Nemerbut et al. 2007, Schmidt et al. 2009). Recent work analyzing the broader microbial communities in other extreme environments, such as Antarctic cryoconite holes and high-elevation soils of the Atacama region, also point to bacteria not necessarily being the primary components of the microbial communities in extreme environments (Costello et al. 2009, Solon et al. 2014). In the present study, bacterial diversity and biogeochemical functioning increased nearly exponentially along the chronosequence (Fig. 1B).
For example, Sommers et al. (2018) found a strikingly high correlation between bacterial and eukaryotic community assembly in cryoconite holes along an extreme nutrient-limitation gradient in the McMurdo Dry Valley of Antarctica. Because previous studies of the bacterial community of the youngest soils of the Puca Glacier showed a predominantly photoautotrophic bacterial community and an abundance of bacteria in the Comamonadaceae (Nemerget al. 2007, Schmidt et al. 2008, Knelman et al. 2014), we expected to see mostly bacterial-feeding nematodes in the youngest soils. However, the dominance of fungal-feeding nematodes (rather than bacterial feeders) in the youngest soils suggests

Fig. 5. GDM models for microbial communities. GDM was fit to community distance for each of our four microbial subsets: (A) bacteria, (B) fungi, (C) nematodes, and (D) other eukaryotes. Scatter plots show how well GDM fit to the data, with observed community distance plotted against GDM’s predicted community distance, and 1:1 lines shown in black ($R^2$ is for 1:1 line). Beneath each scatter plot are GDM i-splines for variables in the model. These splines are partial regression fits, and the shape of the spline reflects the rate at which community composition changes (y-axis) with increasing dissimilarity in that variable (x-axis). All variables are scaled in this plot such that minimum dissimilarity observed is zero and maximum dissimilarity observed is 1. The maximum heights of i-splines reflect the magnitude of total compositional change associated with that variable, for example, the variable’s relative importance to the model. PHOS (blue) was the most important variable for both (A) bacteria and (D) other eukaryotes (B) and was important for (B) fungi and (C) nematodes as well, especially at smaller differences in PHOS activity.

2018, Sommers et al. 2018). For example, Sommers et al. (2018) found a strikingly high correlation between bacterial and eukaryotic community assembly in cryoconite holes along an extreme nutrient-limitation gradient in the McMurdo Dry Valley of Antarctica.
that there may be a significant fungal biomass to support nematodes in these young soils. It was also surprising that fungal guild analyses which showed a high relative abundance of putatively lichenized fungi in the youngest soils. If these fungi are actually lichenized, they are probably associated with cyanobacteria since there were few algal sequences detected in the youngest soils. Any fungal-cyanobacterial association at this site still awaits a direct confirmation. However, based on their dominance, ascomycete Verrucariaceae and cyanobacterial *Nostoc* are the most likely candidates as both have been recognized as lichen partners, particularly in alkaline post-glacial soils (Blundon and Dale 1990, Zhang et al. 2016). These findings show why an analysis of the whole microbial community is needed if we are to begin to understand the microbial ecology and biogeochemistry of early successional soils. It should be noted that there were no lichens nor any other macroscopic structures visible in the 9-year-old sites at the time of sampling. Obviously, more in depth work is needed (including an analysis of lichens and explicit quantification of bacterial and fungal biomass) to elucidate the role of fungi in the youngest soils of the Puca and other high-elevation chronosequences.

Despite the parallel patterns of microbial diversity increase along this chronosequence (Fig. 3), the magnitude of increases in diversity was higher for all of the eukaryotic groups compared with bacteria. That is, the rate at which richness increased along the chronosequence was much lower for bacteria (twofold) than the other groups studied (threefold for other eukaryotes, fourfold for fungi, and 10-fold for nematodes). In fact, nematode communities in 9- and even 24-year-old soils supported predominantly fungal-feeding species (as discussed above). Likewise, other metazoans (e.g., tardigrades, springtails, and enchytraeids) were only detected in the 89-year-old soils suggesting that assembly of soil communities at higher trophic levels is slow at this high-elevation site and that fully functioning higher trophic-level interactions are not occurring during at least the first 24 yr following glacial retreat at this site. This finding emphasizes the extreme nature of the Puca Glacier site compared with other chronosequences at which soil animals have been studied. For example, even at the high-latitude (78.9°N) proglacial foreland of the Midtre Lovénbreen Glacier in Svalbard, multiple detritivore/omnivore microarthropod taxa were already present in 2-year-old soils (Hodkinson et al. 2004).

The present study also adds to our understanding of how nutrient limitation may be controlling patterns of microbial diversity along cold-dry environments such as the Puca chronosequence. Indeed, our measurements of enzyme stoichiometries along this chronosequence indicate extreme P limitation and a moderate level of C limitation across the entire chronosequence (Appendix S1: Fig. S4). These new findings support previous soil microcosm studies and a 6-yr field-fertilization study that also indicated strong P limitation at this site (Darcy et al. 2018). In the present study, the activity of soil phosphatases (PHOS) was more than an order of magnitude higher than the combined activities of N-processing enzymes (NAG and LAP in Appendix S1: Table S2) pointing to P limitation as has been observed at other cold-dry sites (Schmidt et al. 2016, Bueno de Mesquita et al. 2019). PHOS also was the strongest predictor of community composition for both bacteria and other eukaryote communities and was a significant predictor of fungal and nematode communities as well (Fig. 5). Thus, it is likely that P limitation, rather than a harsh climate per se is the primary factor slowing the development of multiterrrophic diversity along the Puca chronosequence.

Despite indications of severe P limitation along the chronosequence, all measures of C and N increased along the chronosequence. In fact, the oldest soils had an order of magnitude higher values of C and N than the youngest soils. Microbial C and N exhibited a parallel pattern, indicating a buildup of microbial biomass as soils aged and supporting the ecological principle that more productive ecosystems support higher biodiversity (Waide et al. 1999, Geyer et al. 2017, Porazinska et al. 2018). This positive relationship in the system studied here appears to partially result from facilitation of microbial processes and taxa through the course of succession. For instance, in the youngest soils with limited C and N resources, the dominant microbes included autotrophic and N-fixing cyanobacteria and lichenized fungi, taxa that not only are able to exist
under extreme oligotrophy but also add C and N to the system which allows colonization of the site by higher trophic groups such as nematodes. It is also likely that taxa at higher trophic levels (e.g., microbial grazers) contribute to a buildup of N pools since they require less N than their prey provides (Ferris et al. 1997, Chen and Ferris 1999) and therefore release N from bacterial and fungal biomass that can be used for algae and plants growth. This is also reflected in the molar C-to-N ratios of the microbial biomass that start out at 10.9 in the 9-year-old soils and decline to 8.4 and 8.9 in the 24- and 89-year-old soils, respectively. The C:N of the biomass in the 24- and 89-year-old soils are in line with the global mean of microbial biomass in vegetated soils of 8.6 (Cleveland and Liptzin 2007), whereas the 9-year-old soils show a slight excess of C in the biomass compared with N.

In addition to biogeochemistry and diversity, there were some significant shifts in apparent microbial functional groups along the gradient. For instance, cyanobacteria (e.g., *Nostoc* and *Microcoleus*) were dominant in the youngest soils but declined as soils aged. In contrast, many potentially N-fixing species within Rhizobiales known to be symbiotic with plants (e.g., *Bradyrhizobium*, *Microvirga*, and *Rhizobium*) increased in abundance with the age of soils. Among fungi, the most dominant taxa in the youngest soils were identified as lichenized fungi (mostly from ascomycete Verrucariaceae that accounted for more than 50% of the fungal community) and saprotrophs (e.g., *Rhinocladiella* and *Tetracladium*). In contrast, plant parasites were present only in older (especially 89-year-old) soils and endomycorrhizal fungi only in the oldest (89-yr) soils. Colonization of the youngest soils (9-year-old) was limited to fungal-feeding nematode species, whereas the oldest soils contained other feeding groups (e.g., a bacterial-feeding *Rhabdolaimus* and an omnivorous/predatory *Nygolaimus*). The unexpected early predominance of fungal-feeding nematodes suggests a significant fungal biomass must reside in these soils. It appears that lichenized fungi could be the most likely candidate food source for fungal-feeding nematodes.

It is important to note that functional traits assigned to taxa may be imprecise in some cases. First, relatively short length of sequences can be insufficiently informative to uncover the exact identity of microbial species (Clarke et al. 2017, Ahmed et al. 2019). Second, identified taxa within all soil groups were rarely exact matches to known reference sequences. For instance, among a total of 68 nematode species only two produced 100% hits. Third, many taxa were simply unknown. For instance, a large component of fungal communities, regardless of their successional stage, was unidentifiable even at the class level not only with the use of databases specifically designed for fungi (e.g., UNITE), but also databases that are more inclusive and updated more frequently (e.g., NCBI). And fourth, functional traits of most microbial taxa are largely unknown. For example, while FUNGuild database classifies *Exophiala* as animal pathogens, a closer analysis shows that our sequences are not related to any animal pathogens. Many *Exophiala* have been recently shown to be DSE (dark septate endophytes) of alpine plant roots, and the closest BLAST matches to our *Exophiala* sequences were all from studies of plant roots or from rhizosphere soils. For example, one of our *Exophiala* ASVs was 100% identical to endophytic and rhizosphere fungi from roots of *Paonia ludlowii* in Tibet (MK983935) and to montane pines in Montenegro (Lazarević and Menkis 2018) and a 99% match to high-elevation root endophytes from Colorado (Bueno de Mesquita et al. 2018). Likewise, another *Exophiala* ASV was a 98% match to Colorado root endophytes (Bueno de Mesquita et al. 2018), and another was a 97% match to phylosphere fungi of European Beech (Cordier et al. 2012). Also, many *Exophiala* are Black Yeasts and are among the most abundant fungal phylotypes in sequencing studies of the soils of the Dry Valleys of Antarctica (Dreesens et al. 2014), and in desert crusts of the western USA (Bates et al. 2006). Therefore, the *Exophiala* sequences found in our study are likely root endophytes or perhaps crust associated fungi, but they are unlikely to be related to animal pathogens as they are classified in FUNGuild.

Finally, while a minimal number of samples were used for inference (four replicates at three distances for a total of 12 samples), they were sufficient to illustrate the key patterns reported here. Each of the 12 collected samples represented three homogenized subsamples capturing potential spatial heterogeneity on the scale of
less than one meter. Moreover, the four replicate composite samples representative of each soil age category were sampled to minimize autocorrelation and maximize the integration of spatial variation (King et al. 2010). Despite inherent differences in natural variation among investigated biotic groups, the observed diversity patterns for all groups were consistently similar, with statistical results producing a robust and coherent story. And finally, the results from this research were very much in agreement with results from previous research from the Puca Glacier chronosequence. In all, despite the shallow sampling, the analytical approaches used in this study provide for high confidence in the validity of the results.

CONCLUSIONS

We investigated all major soil groups including bacteria, fungi, nematodes, and other eukaryotes along the Puca Glacier chronosequence spanning 9- to 89-year-old soils. Here, we show that contrary to our expectations: (1) All of the microbial soil groups showed similar diversity patterns along this chronosequence with all groups becoming more diverse and more complex as soil became older, (2) the fungal component of the community was likely well established early in succession as indicated by the dominance of fungal-feeding (not bacterial-feeding) nematodes in the youngest soils, and (3) all soil groups were highly correlated with soil biogeochemical characteristics indicating that at these early stages of primary succession all of the various soil groups are really members of the same developing community.

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LITERATURE CITED

Ahmed, M., M. A. Back, T. Prior, G. Karssen, R. Lawson, I. Adams, and M. Sapp. 2019. Metabarcoding of soil nematodes: the importance of taxonomic coverage and availability of reference sequences in choosing suitable markers. Metabarcoding and Metagenomics 3:e36408.

Aitchison, J., C. Barcelo-Vidal, J. A. Martin-Fernandez, and V. Pawlowsky-Glahn. 2000. Logratio analysis and compositional distance. Mathematical Geology 32:271–275.

Amaral-Zettler, L. A., E. A. McCliment, H. W. Ducklow, and S. M. Huse. 2009. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. PLOS ONE 4:e6372.

Bates, S. T., G. S. N. Reddy, and F. Garcia-Pichel. 2006. *Exophiala crusticola* anam. nov. affinity Herpotrichiellaceae, a novel black yeast from biological soil crusts in the Western United States. International Journal of Systematic and Evolutionary Microbiology 56:2697–2702.

Bellemain, E., T. Carlsen, C. Brochmann, E. Coissac, P. Taberlet, and H. Kauserud. 2010. ITS as an environmental DNA barcode for fungi: An in-silico approach reveals potential PCR biases. BMC Microbiology 10:189.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate – a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B-Statistical Methodology 57:289–300.

Bernard, G. C., M. Egnin, and C. Bonsi. 2017. The impact of plant-parasitic nematodes on agriculture and methods of control. Pages 112–151 in M. M. Shah and M. Mamood, editors. Nematology – Concepts, diagnosis and control. InTech, Rijeka, Croatia.

Blaalid, R., T. Carlsen, S. Kumar, R. Halvorsen, K. I. Ugland, G. Fontana, and H. Kauserud. 2012. Changes in the root-associated fungal communities along a primary succession gradient analysed by 454 pyrosequencing. Molecular Ecology 21:1897–1908.

Blundon, D. J., and M. R. T. Dale. 1990. Dinitrogen fixation (acetylene reduction) in primary succession near Mount Robson, British Columbia, Canada. Arctic and Alpine Research 22:255–263.

Bolyen, E., et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37:852–857.
Bongers, T., and H. Ferris. 1999. Nematode community structure as a bioindicador in environmental monitoring. Trends in Ecology & Evolution 14:224–228.

Bradley, J. A., J. S. Singarayer, and A. M. Aneiso. 2014. Microbial community dynamics in the forefield of glaciers. Proceedings of the Royal Society B: Biological Sciences 281:20140882.

Brown, S. P., and A. Jumpponen. 2014. Contrasting primary successional trajectories of fungi and bacteria in retreating glacier soils. Molecular Ecology 23:481–497.

Bueno de Mesquita, C. P., L. M. Brigham, P. Sommers, D. L. Porazinska, E. C. Farrer, A. J. King, M. J. Spasojevic, J. G. Smith, K. N. Suding, and S. K. Schmidt. 2018. Patterns of root colonization by arbuscular mycorrhizal fungi and dark septate endophytes across a mostly-unvegetated, high-elevation landscape. Fungal Ecology 36:63–74.

Callahan, B. J., P. J. McMurdie, and S. P. Holmes. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME Journal 11:2639–2643.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nature Methods 13:581–583.

Camacho, C., G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, and T. L. Madden. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421.

Caporaso, J. G., et al. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336.

Caporaso, J. G., et al. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME Journal 6:1621–1624.

Castle, S. C., D. R. Nemergut, A. S. Grandy, J. W. Leff, E. B. Graham, E. Hood, S. K. Schmidt, K. Wickings, and C. C. Cleveland. 2016. Biogeochemical drivers of microbial community convergence across actively retreating glaciers. Soil Biology and Biochemistry 101:74–84.

Castle, S. C., B. W. Sullivan, J. Knelman, E. Hood, D. R. Nemergut, S. K. Schmidt, and C. C. Cleveland. 2017. Nutrient limitation of soil microbial activity during the earliest stages of ecosystem development. Oecologia 185:513–524.

Chen, J., and H. Ferris. 1999. The effects of nematode grazing on nitrogen mineralization during fungal decomposition of organic matter. Soil Biology and Biochemistry 31:1265–1279.

Clarke, L. J., J. M. Beard, K. M. Swadling, and B. E. Deagle. 2017. Effect of marker choice and thermal cycling protocol on zooplankton DNA metabricoding studies. Ecology and Evolution 7:873–883.

Cleveland, C. C., and D. Liptzin. 2007. CN: P stoichiometry in soil: Is there a Redfield ratio for the microbial biomass? Biogeochemistry 85:235–252.

Cordier, T., C. Robin, X. Capdevielle, O. Fabreguettes, M. L. Desprez-Loustau, and C. Vacher. 2012. The composition of phyllosphere fungal assemblages of European beech (Fagus sylvatica) varies significantly along an elevation gradient. New Phytologist 196:510–519.

Costello, E. K., S. R. P. Halloy, S. C. Reed, P. Sowell, and S. K. Schmidt. 2009. Fumarole-supported islands of biodiversity within a hyperarid, high-elevation landscape on Socompa Volcano, Puna de Atacama, Andes. Applied Environmental Microbiology 75:735–747.

Cutler, N. A., D. L. Chaput, and C. J. van der Gast. 2014. Long-term changes in soil microbial communities during primary succession. Soil Biology and Biochemistry 69:359–370.

Darcy, J. L., S. K. Schmidt, J. E. Knelman, C. C. Cleveland, S. C. Castle, and D. R. Nemergut. 2018. Phosphorus, not nitrogen, limits plants and microbial primary producers following glacial retreat. Science Advances 4:aaq0942.

De Caceres, M., and M. P. Legendre. 2009. Associations between species and groups of sites: indices and statistical inference. Ecology 90:3566–3574.

Dreesens, L., C. Lee, and S. Cary. 2014. The distribution and identity of edaphic fungi in the McMurdo Dry Valleys. Biology 3:466–483.

Egozcue, J. J., V. Pawlowsky-Glahn, G. Mateu-Figueras, and C. Barcelo-Vidal. 2003. Isometric logratio transformations for compositional data analysis. Mathematical Geology 35:279–300.

Ferrier, S., G. Manion, J. Elith, and K. Richardson. 2007. Using generalized dissimilarity modelling to analyse and predict patterns of beta diversity in regional biodiversity assessment. Diversity and Distributions 13:252–264.

Ferris, H., R. C. Venette, and S. S. Lau. 1997. Population energetics of bacterial-feeding nematodes: carbon and nitrogen budgets. Soil Biology and Biochemistry 29:1194–1198.

Fitzpatrick, M. C., and S. R. Keller. 2015. Ecological genomics meets community-level modelling of biodiversity: mapping the genomic landscape of
current and future environmental adaptation. Ecology Letters 18:1–16.

Geyer, K. M., C. D. Takacs-Vesbach, M. N. Gooseff, and J. E. Barrett. 2017. Primary productivity as a control over soil microbial diversity along environmental gradients in a polar desert ecosystem. PeerJ 5:e3377.

Hill, B. H., C. M. Elonen, L. R. Seifert, A. A. May, and E. Tarquini. 2012. Microbial enzyme stoichiometry and nutrient limitation in US streams and rivers. Ecological Indicators 18:540–551.

Hodda, M., L. Peters, and W. Traunspurger. 2009. Nematode diversity in terrestrial, freshwater aquatic and marine systems. Pages 45–93 in M. J. Wilson and T. Kakouli-Duarte, editors. Nematology: Advances and perspectives. CABI, Wallingford, Oxfordshire, UK.

Hodkinson, I. D., S. J. Coulson, and N. R. Webb. 2004. Invertebrate community assembly along proglacial chronosequences in the high Arctic. Journal of Animal Ecology 73:556–568.

Jiang, Y. L., Y. B. Lei, Y. Yang, H. Korpelainen, U. Niinemets, and C. Y. Li. 2018. Divergent assemblage patterns and driving forces for bacterial and fungal communities along a glacier forefield chronosequence. Soil Biology and Biochemistry 118:207–216.

Kim, M., J. Y. Jung, D. Laffly, H. Y. Kwon, and Y. K. Lee. 2017. Shifts in bacterial community structure during succession in a glacier foreland of the High Arctic. FEMS Microbiology Ecology 93:fiw213.

King, A. J., K. R. Freeman, K. F. McCormick, R. C. Lynch, C. Lozupone, R. Knight, and S. K. Schmidt. 2010. Biogeography and habitat modelling of high-alpine bacteria. Nature Communications 1:53.

Knelman, J. E., S. K. Schmidt, R. C. Lynch, J. L. Darcy, S. C. Castle, C. C. Cleveland, and D. R. Nemergut. 2014. Nutrient addition dramatically accelerates microbial community succession. PLOS ONE 9: e102609.

Lambshead, P. J. D. 2004. Marine nematode biodiversity. Pages 438–468 in Z. X. Chen, S. Y. Chen, and D. W. Dickson, editors. Nematology: Advances and perspectives. CABl, Wallingford, Oxfordshire, UK.

Larsson, J. 2020. Eulerr: Area-proportional euler and venn diagrams with ellipses. R package version 6.1.0. https://cran.r-project.org/package=eulerr

Lazarević, J., and A. Menkis. 2018. Fungi inhabiting fine roots of Pinus heldreichii in the Montenegrin montane forests. Symbiosis 74:189–197.

Legendre, P., and M. J. Oksanen. 2018. imodel2: Model II regression. R package version 1.7-3. https://CRAN.R-project.org/package=imodel2

Manion, G., M. Lisk, S. Ferrier, D. Nieto-Lugilde, and M. Fitzpatrick. 2016. gdm: functions for generalized dissimilarity modeling. R package. https://cran.r-project.org/web/packages/gdm/index.html

Marshall, S. 2014. Glacier retreat crosses a line. Science 345:872.

McMurdie, P. J., and S. Holmes. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLOS ONE 8: e61217.

Nemergut, D. R., S. P. Anderson, C. C. Cleveland, A. P. Martin, A. E. Miller, A. Seimon, and S. K. Schmidt. 2007. Microbial community succession in an unvegetated, recently deglaciated soil. Microbial Ecology 53:110–122.

Nguyen, N. H., Z. W. Song, S. T. Bates, S. Branco, L. Tedersoo, J. Menke, J. S. Schilling, and P. G. Kennedy. 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20:241–248.

Nilsson, R. H., et al. 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research 47:D259–D264.

Oksanen, J., et al. 2011. Vegan: community ecology package version 2.0-2. R CRAN package. https://cran.r-project.org/web/packages/vegan/index.html

Porazinska, D. L., E. C. Farrer, M. J. Spasojevic, C. P. Bueno de Mesquita, S. A. Sartwell, J. G. Smith, C. T. White, A. J. King, K. N. Suding, and S. K. Schmidt. 2018. Plant diversity and density predict belowground diversity and function in an early successional alpine ecosystem. Ecology 99:1942–1952.

Pruesse, E., C. Quast, K. Knittel, B. M. Fuchs, W. Ludwig, J. Peplies, and F. O. Glockner. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Research 35:7188–7196.

R Development Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.Rproject.org/

Rousk, J., E. Baath, P. C. Brookes, C. L. Lauber, C. Lozupone, J. G. Caporaso, R. Knight, and N. Fierer. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME Journal 4:1340–1351.

Schaaf, W., et al. 2011. Patterns and processes of initial terrestrial-ecosystem development. Journal of Plant Nutrition and Soil Science 174:229–239.

Schmidt, S. K., et al. 2008. The earliest stages of ecosystem succession in high-elevation (5000 metres
above sea level), recently deglaciated soils. Proceedings of the Royal Society B: Biological Sciences 275:2793–2802.

Schmidt, S. K., D. R. Nemergut, J. L. Darcy, and R. Lynch. 2014. Do bacterial and fungal communities assemble differently during primary succession? Molecular Ecology 23:254–258.

Schmidt, S. K., D. R. Nemergut, A. E. Miller, K. R. Freeman, A. J. King, and A. Seimon. 2009. Microbial activity and diversity during extreme freeze-thaw cycles in periglacial soils, 5400 m elevation, Cordillera Vilcanota, Perú. Extremophiles 13:807–816.

Schmidt, S. K., D. L. Porazinska, B. L. Concienne, J. L. Darcy, A. J. King, and D. R. Nemergut. 2016. Biogeochemical stoichiometry reveals P and N limitation across the post-glacial landscape of Denali National Park, Alaska. Ecosystems 19:1164–1177.

Schmidt, S. K., and L. Vimercati. 2019. Growth of cyanobacterial soil crusts during diurnal freeze-thaw cycles. Journal of Microbiology 57:243–251.

Schratzberger, M., M. Holterman, D. van Oevelen, and J. Helder. 2019. A worm’s world: Ecological flexibility pays off for free-living nematodes in sediments and soils. BioScience 69:867–876.

Seimon, T. A., A. Seimon, P. Daszak, S. Halloy, L. Schloegel, C. Aguilar, P. Sowell, A. Hyatt, B. Konecky, and J. Simmons. 2007. Upward range extension of Andean anurans and chytridiomycosis to extreme elevations in response to deglaciation. Global Change Biology 13:288–299.

Sinsabaugh, R. L., B. H. Hill, and J. J. F. Shah. 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462:795–798.

Solon, A. J., L. Vimercati, J. L. Darcy, P. Arian, D. L. Porazinska, C. Dorador, M. E. Farias, and S. K. Schmidt. 2018. Microbial communities of high-elevation fumaroles, penitents and dry tephra “soils” of the Punta de Atacama Volcanic Zone. Microbial Ecology 5:1–12.

Sommers, P., J. L. Darcy, E. M. Gendron, L. F. Stanish, E. A. Bagshaw, D. L. Porazinska, and S. K. Schmidt. 2018. Diversity patterns of microbial eukaryotes mirror those of bacteria in Antarctic cryoconite holes. FEMS Microbiology Ecology 94:fix167.

Waide, R. B., M. R. Willig, C. F. Steiner, G. Mittelbach, L. Gough, S. I. Dodson, G. P. Juday, and R. Parminter. 1999. The relationship between productivity and species richness. Annual Review of Ecology and Systematics 30:257–300.

Weintraub, M. N., L. E. Scott-Denton, S. K. Schmidt, and R. K. Monson. 2007. The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in a subalpine forest ecosystem. Oecologia 154:327–338.

Yeates, G. W., T. Bongers, R. G. M. De Goede, D. W. Freckman, and S. S. Georgieva. 1993. Feeding habits in soil nematode families and genera—an outline for soil ecologists. Journal of Nematology 25:315–331.

Zhang, T., N. F. Wang, H. Y. Liu, Y. Q. Zhang, and L. Y. Yu. 2016. Soil pH is a key determinant of soil fungal community composition in the Ny-Alesund Region, Svalbard (High Arctic). Frontiers in Microbiology 4:1072–1083.

DATA AVAILABILITY

All data including raw sequences and mapping files have been deposited at NCBI with the accession number: PRJNA594971, https://www.ncbi.nlm.nih.gov/sra/?term=prjna594971.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3400/full