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Contrasting primary successional trajectories of fungi and bacteria in retreating glacier soils

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Abstract

Early community assembly of soil microbial communities is essential for pedogenesis and development of organic legacies. We examined fungal and bacterial successions along a well-established temperate glacier forefront chronosequence representing ~70 years of deglaciation to determine community assembly. As microbial communities may be heavily structured by establishing vegetation, we included nonvegetated soils as well as soils from underneath four plant species with differing mycorrhizal ecologies (*Abies lasiocarpa*, ectomycorrhizal; *Luetkea pectinata*, arbuscular mycorrhizal; *Phyllodoce empetriformis*, ericoid mycorrhizal; *Saxifraga ferruginea*, nonmycorrhizal). Our main objectives were to contrast fungal and bacterial successional dynamics and community assembly as well as to decouple the effects of plant establishment and time since deglaciation on microbial trajectories using high-throughput sequencing. Our data indicate that distance from glacier terminus has large effects on biomass accumulation, community membership, and distribution for both fungi and bacteria. Surprisingly, presence of plants rather than their identity was more important in structuring bacterial communities along the chronosequence and played only a very minor role in structuring the fungal communities. Further, our analyses suggest that bacterial communities may converge during assembly supporting determinism, whereas fungal communities show no such patterns. Although fungal communities provided little evidence of convergence in community structure, many taxa were nonrandomly distributed across the glacier foreland; similar taxon-level responses were observed in bacterial communities. Overall, our data highlight differing drivers for fungal and bacterial trajectories during early primary succession in recently deglaciated soils.

Keywords: 454 sequencing, bacteria, fungi, glacier forefront, primary succession, rhizosphere

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Introduction

Primary succession has been studied in a number of North American temperate ecosystems and, consequently, much is known about plant establishment in volcanic (del Moral & Bliss 1993; del Moral *et al.* 1995) and glacier foreland systems (Chapin *et al.* 1994; Cázares *et al.* 2005; Jumpponen *et al.* 2012). In contrast, studies on microbial successional dynamics in these systems remain relatively few. With the growing concern of accelerating glacier recession (Barry 2006) and potential changes in global biogeochemical patterns (Cramer *et al.* 2001), it is becoming increasingly important to understand the fundamentals of microbial successional dynamics. Microbial primary succession has often been studied via rRNA community fingerprinting or cloning and sequencing (Sigler & Zeyer 2002; Jumpponen 2003), ectomycorrhizal root tip analysis (Nara *et al.* 2003; Trowbridge & Jumpponen 2004), and biochemical assays of soils and associated organisms (Ohtonen *et al.* 1999; Tscherko *et al.* 2005). With the advancement of high-throughput sequencing, these microbial communities in primary successional environments can now be queried in greater depth for both fungi (Blaalid *et al.* 2012) and bacteria (Schütte *et al.* 2010). Such studies have elucidated some general patterns of community dynamics over successional age. However, these

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patterns have not been explored simultaneously for both bacteria and fungi using high-throughput sequencing assays. Consequently, comparisons of fungal and bacterial successional dynamics are rare and preclude elucidation of universal patterns of microbial succession in soils. Concurrent examination of fungi and bacteria is mandatory to understand if these microbial guilds adhere to similar rules. Additionally, such studies permit evaluation of whether general conclusions or conceptual frameworks derived from plant community succession apply also to microbial communities.

Microbial succession does not occur in a vacuum. Rather, microbial communities interact with allochthonous substrates as well as early establishing autotrophs. Although essential to our greater appreciation of the microbial succession, the effects of substrate age and plant establishment remain insufficiently decoupled. This is especially true for plants whose mycorrhizal habits can be presumed to select particular fungal symbionts from the available propagule pools (Jumpponen & Egerton-Warburton 2005). These organismal interactions probably have precipitous effects across the establishing soil communities outside symbiotic partnerships. Previous studies on fungal community dynamics in early primary successional environments have often focused solely on one mycorrhizal habit: ericoid (Tejesvi *et al.* 2010), ecto- (Helm *et al.* 1996; Trowbridge & Jumpponen 2004; Ashkannejhad & Horton 2006; Mühlmann & Peintner 2008) or arbuscular (Oehl *et al.* 2011; Sikes *et al.* 2012) mycorrhizas. As a result, a greater understanding of how vegetation may drive the microbial community succession can be gained through broader inclusion of mycorrhizal habits. Similarly, analyses of bacterial primary succession have focused on successional age (Schütte *et al.* 2010), or to a limited extent, vegetation influencing microbial communities (Knelman *et al.* 2012) and biogeochemical processes (Schmidt *et al.* 2008). Few previous studies investigated joint effects of successional age and vegetation establishment on microbial dynamics (Ohtonen *et al.* 1999; Zumsteg *et al.* 2012).

To our knowledge, this study represents one of the very first concurrent analyses of bacterial and fungal primary successional dynamics while also accounting for the effects of substrate age and establishment of plants that represent a broad selection of mycorrhizal habits utilizing deep interrogation of the soil microbial communities afforded by next-generation locus-targeted sequencing. In the studies reported here, we evaluated the fungal and bacterial community composition using direct 454-pyrosequencing of ribosomal RNA (rRNA) gene targets. Our overall goal was to simultaneously analyse community dynamics of bacteria and fungi in a retreating glacier forefront system that is currently undergoing primary succession. We tested hypotheses on how time since deglaciation and established plants with differing mycorrhizal habits affect the fungal and bacterial communities during early primary succession. Specifically, we hypothesized that: (i) microbial communities increase in richness and diversity as substrate ages and becomes more heterogeneous as a result of plant establishment, (ii) microbial communities associated with plants with different mycorrhizal habits are distinct, (iii) fungal and bacterial biomasses increases with successional age and shift from a bacteria-dominated early successional system to a fungus-dominated late successional system as plant establishment becomes of increasing importance, (iv) communities exhibit successional trajectories as substrate ages indicating deterministic processes over successional time with specific plant-associated mycorrhizal ecologies differentially influencing such deterministic patterns. These hypotheses are based partly on those derived for plant communities and summarized for comparable systems in Jumpponen *et al.* (2012).

Material and methods

Study site

Lyman Glacier (~1900 m a.s.l) is located within the Wenatchee National Forest in the North Cascade Mountain range in Washington State, USA. (48°10′14″N, 120°53′44″W). Its receding forefront is characterized by a well-documented chronosequence of approximately a century with the terminal moraine located 1100 m north of the glacier terminus and suited well for a space for time substitution (Jumpponen *et al.* 1998). Plant (Cázares 1992; Jumpponen *et al.* 2012) and ectomycorrhizal fungus (Jumpponen *et al.* 1999, 2002; 2012) communities as well as root colonization of various plant hosts (Trowbridge & Jumpponen 2004; Cázares *et al.* 2005) have been previously characterized at this site.

Sampling

Topsoil (0–5 cm) samples were collected from nonvegetated bare soils as well as from the rhizospheres of four plant species differing in their mycorrhizal habits along the chronosequence at 150-m intervals ranging from 0 to 750 m from the glacier terminus (representing 0 to ~70 years since deglaciation). In all, we analysed 72 samples (3 at 0 m, 9 at 150 m, 15 each at 300, 450, 600 and 750 m from the glacier terminus). The target plant species occur commonly along the chronosequence and included ectomycorrhizal (EcM) *Abies lasiocarpa* (Hook.) Nutt., arbuscular mycorrhizal (AM) *Luetkea pectinata* Kuntze, ericoid mycorrhizal (ErM) *Phyllodoce*

empetriformis D.Don, and nonmycorrhizal (NM) *Saxifraga ferruginea* Graham. Plant root colonization according to their mycorrhizal habits was earlier confirmed at this site (Cázares *et al*. 2005). From here on, these plants will be referred to only by their genus. Plant-associated soils were dug from the centre of vegetation patch or directly underneath *Abies* stem; *Saxifraga*-associated soils were sampled by excavating the whole plant and the associated soil collected. The three replicate samples for each plant or barren soil within each 150-m interval were collected at least 5 m apart. Nonvegetated soils were collected at least 1 m from any established vegetation to ensure absence of roots in the substrate. Soils were sieved through a 5-mm mesh to remove large rocks and root fragments, manually homogenized, and two subsamples (~350 μL; 0.57 g ± 0.15) collected directly into two DNA extraction bead tubes (UltraClean Soil DNA Isolation kit; MoBio, Carlsbad, CA, USA). The extraction tubes were placed on ice in collapsible coolers and shipped to the laboratory at Kansas State University within 72 h, where frozen at −20 °C upon arrival. The two subsamples were treated independently through DNA extraction and PCR amplification. A third soil sample was collected into a 2-mL collection tube and used to determine soil dry weight. All samples were collected on 8th September 2009.

DNA extraction and analysis

DNA was extracted using UltraClean Soil DNA Isolation kits following the manufacturer's standard protocol. Extracted DNA was quantified using an ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and template DNA for each sample was aliquoted into 96-well plates at a working concentration of 2.5 ng/μL.

PCR amplicons were generated for 454 sequencing using Lib-L unidirectional emPCR kit (Roche Applied Science, Indianapolis, IN, USA). Fungal amplicons of the internal transcribed spacers 1 and 2 (ITS1 and ITS2) were generated using an 8-bp DNA-bar-coded forward primer A-ITS1f (A-MIDs-CTTGGTCATTTAGAGGAAGTAA) and reverse primer of B-ITS4 (B-TCCTCCGCTTATTGATATGC) as described in the study by Jumpponen *et al.* (2010). Bacterial amplicons were generated using a forward B-9F (B-GAGTTTGATCMTGGCTCAG) primer and 8-bp DNA-bar-coded [see Table S1 (Supporting Information) for complete list of MID sequences] reverse primer A-541R (A-MIDs-WTTACCGCGGCTGCTGG; Muyzer *et al.* 1993) to amplify V1 and V2 regions of the 16s rRNA (Baker *et al.* 2003). Each subsample was amplified in three independent 25 μL PCRs for technical replication. PCR conditions for fungi were 10 μM forward and reverse primers, 5 ng template DNA, 200 μM of each dioxynucleotide, 25 mM $MgCl_2$, 5 μL 5× Green GoTaq® Flexi Buffer (Promega, Madison, WI, USA), 7.8 μL molecular biology grade water and 1 U GoTaq® Hot Start Polymerase (Promega). PCR cycle parameters consisted of a 94 °C initial denaturing for 3 min, four cycles of step-down PCR at 94 °C denaturing for 1 min, 57–54 °C of annealing for 1 min, and 72 °C extension for 2 min followed by 29 cycles of 94 °C denaturing for 1 min, 54 °C annealing for 1 min and 72 °C extension for 2 min followed by 8 min 72 °C final extension. PCR conditions for bacteria were 10 μM forward and reverse primers, 5 ng template DNA, 12.5 μL AmpliTaq Gold® Master Mix (Applied Biosystems, Foster City, CA, USA), 5.5 μL molecular biology grade water with PCR cycle parameters with an initial denaturing step of 95 °C for 4 min, followed by 27 cycles of 95 °C denaturing for 30 s, 54 °C annealing for 1 min and 72 °C extension for 2 min, followed by a final extension step of 72 °C for 8 min 30 s. All PCRs were performed on MasterCyclers (Eppendorf, Hamburg, Germany). Negative controls for DNA extractions and PCRs were included to ensure absence of contamination; no contamination was detected on PCR products visualized via an agarose gel electrophoresis.

Each separate PCR (3 technical replicates, 2 subsamples) was visualized on a 1.5% agarose (*w*/*v*) gel to ensure presence of PCR products. One fungal sample was omitted from further analysis because no product could be obtained. The remaining volume (20 μL) of the six replicate PCR amplicons per experimental unit was pooled and cleaned with Agencourt® AmPure® cleanup kit using an SPRIplate 96-ring magnet (Beckman Coulter, Beverly, MA, USA) following the manufacturer's protocol with the exception that we used a 1:1 ratio of bead solution to reaction volume to discriminate against nontarget small DNA fragments. Purified amplicons were quantified with NanoDrop and amplicons pooled at equal molarity (336 ng per fungal sample and 294 ng per bacterial sample) to equally represent each sample in subsequent sequencing reaction. Pooled samples were visualized on a low-melt agarose gel and the target-sized amplicon (~600–800 bp for fungi and ~500 bp for bacteria) excised for purification with UltraClean™ GelSpin™ DNA Extraction Kit (MoBio). The gel-excised fungal (1109.5 ng in 100 μL) and bacterial (1305 ng in 100 μL) amplicons were 454-pyrosequenced (GS FLX Titanium, Roche Applied Science) at the Integrated Genomics Facility at Kansas State University (Manhattan, KN, USA). All sequences (.fastq for each experimental unit for fungi and bacteria) are deposited in NCBI Sequence Read Archive under the Accession nos (SRR943164-SRR943301).

The acquired sequence (.fasta) and quality (.qual) files were processed using the PyroTagger pipeline (Kunin & Hugenholtz 2010), where sequences that

lacked an exact match to the MID-Primer sequence or were of poor quality (≥3% of bases with Q-values <27), and/or were of insufficient length (<350 bp) were culled, and sequences that passed quality control were truncated to 350 bp. Operational taxonomic units (OTUs) were assigned at a 97% similarity threshold (UCLUST; Edgar 2010). Singleton OTUs were omitted from analysis as potential artefacts following Tedersoo *et al.* (2010) recommendation. A representative sequence for each fungal OTU was BLAST-n (nr/nt) (NCBI) queried for taxonomic affinity, and matches with largest maximum identity were chosen after omission of uncultured/environmental accessions. Bacterial OTU taxon affinities were determined using Ribosomal Database Project's (RDP) Naïve Bayesian rRNA Classifier (Wang *et al.* 2007) using an 80% bootstrap confidence threshold. Putative mitochondrion and chloroplast sequences were removed from further analysis. The OTUs considered potentially chimeric in PyroTagger were further examined by manually checking a representative sequence of each OTU in BLAST-n (i.e. if the 5′- and 3′-ends of the queries aligned to different matches, the OTUs were culled); 19 fungal OTUs and 135 bacterial OTUs were determined to be putatively chimeric and omitted.

Ribosomal copy number estimates were determined as a proxy for bacterial and fungal biomasses by quantitative PCR (qPCR) (Fierer *et al.* 2005). Briefly, plasmid standards were generated by amplifying *Escherichia coli* with primers EUB 338F (Lane 1991) and 518R (Muyzer *et al.* 1993) and *Agaricus bisporus* with primers ITS1F (Gardes & Bruns 1993) and 5.8S (Vilgalys & Hester 1990). Amplified products were cloned using One Shot® TOP10 Chemically Competent Cells and TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) and plasmids from positive transformants purified with UltraClean® plasmid prep kit (MoBio). Triplicate qPCRs were performed in an iCycler iQ™ (Bio-Rad, Hercules, CA, USA) RT–PCR detection system and the program iCycler (v. 3.1.7050) in 25 μL reactions consisting of 12.5 μL iQ™ SYBR® Green Supermix (Bio-Rad), 1.25 μL (12.5 μM) forward primer, 1.25 μL (12.5 μM) reverse primer, 5 ng template DNA and 8 μL molecular biology grade H_2O. PCR cycle parameters were initial denaturation at 95 °C for 5 min, and 55 cycles of 95 °C denaturation for 1 min, 53 °C annealing step for 30 s, and 72 °C extension step for 1 min. Standard curves for bacteria and fungi were generated using a 10-fold dilution series (4×10^{-7}–4×10^{-3} ng of the plasmid DNA per reaction). Copy numbers per gram dry soil were calculated (Pfaffl 2001) from the standard curves taking into account the length of target region and assuming an average molecular mass of 660 g/mol for double-stranded DNA.

Diversity indices

Operational taxonomic units richness (S) per sample was calculated by summing the number of OTUs. Complement of the Simpson's diversity index ($1\text{-}D = 1 - \Sigma\, p_i^2$) was calculated for each sample where p_i is the frequency that each OTU occurred in each sample. Shannon–Weaver's H′ was also calculated, but was omitted from analyses as the probabilistic nature of Simpson's index is more informative and the two indices were congruent. We also calculated evenness [$E_D = (1/D)/S$)], where S is the OTU richness in each sample and D is Simpson's diversity index. To explore sequence depth across age of succession and vegetation type, rarefaction curves were generated using EstimateS (version 8.0.0; Colwell 2006).

Statistical analysis

To equalize sequencing effort per sample, we used an outlier analysis, in which a sample was omitted from analysis if the number of sequences was outside the range of ± 2SD (approximates a 95% CI assuming normal distribution across the data set) from mean sequence number. Subsequently, three bacterial samples were omitted from all subsequent analyses, whereas all fungal samples were included. Simpson's diversity and evenness were arcsine-transformed prior to analyses to meet the normality assumptions for ANOVA. To address the effects of distance from the glacier terminus and the effects of plant rhizospheres on OTU richness, Simpson's diversity and evenness, linear regression and ANOVA as well as subsequent pairwise comparisons (Tukey's HSD) were performed. In these analyses, we treated distance from the glacier terminus either as a continuous (linear regression) or categorical variable (ANOVA). All statistical analyses were conducted using JMP® (version 7.0.2; SAS Institute, Cary, NC, USA). To examine community composition and clustering of the treatments, Nonmetric Multidimensional Scaling (NMS) multivariate analysis was performed using the Sørensen (Bray–Curtis) dissimilarity matrix in PC-ORD (version 4.1; McCune & Mefford 1999). The optimal number of dimensions (k) was selected based on Monte Carlo tests using empirical data compared with 100 randomized runs with a decrease in dimensionality from six dimensions to one. Based on the decline in stress, we chose $k = 3$ dimensions. The ordination scores for each of the three axes were analysed by linear regression and ANOVA across distance and vegetation as described above. Additionally, axes score standard deviations of vegetation treatments by distance from glacier terminus were tested (ANOVA) to determine convergence or divergence of the

communities (del Moral *et al.* 1995, Jumpponen *et al.* 2012).

The abundance of each OTU was tested for random distribution across the experimental design matrix. If species distribution in a successional environment is dictated purely by random allochthonous propagule input, then, it stands to reason that species distribution would follow a discrete probability function where rarity is expected at any given location, in other words following a distribution such as Poisson. Consequently, for each OTU, we tested if its distribution differed from an expected Poisson distribution using a Chi-squared test ($df = 4$) on a contingency table of observed sequence frequencies per sample and site. Significance was determined after Bonferroni correction (critical value $\alpha = 0.05/n$) where n is the number of taxa tested ($n = 4114$ for bacteria and $n = 310$ for fungi). We used frequency categories for Poisson analysis following an increasing logarithmic scale (0, 1–10, 11–100, 101–1000, 1000+).

Ecosystem processes and successional dynamics are probably driven largely by community members that occur most frequently. For this reason, we identified core taxa (defined as present in ≥50% of samples; see Unterseher *et al.* 2011) for the glacier forefront system as a whole as well as for each of the different treatment categories. We tested these core taxa for changes in occurrence (square root + 0.5 transformed counts) across distance and rhizosphere environments using a combination of linear regression and ANOVA as described above.

Results

Sequence data

To characterize microbial communities in the Lyman Glacier forefront, we 454-sequenced 180 421 fungal and 194 513 bacterial amplicons. After excluding short (350 bp threshold) and poor-quality reads, 39 509 fungal and 97 716 bacterial sequences remained resulting in an average sequencing depth of 539 ± 387 (mean $\pm$ SD) per sample for fungi and 1454 ± 391 per sample for bacteria. The number of fungal sequences was invariable across distance and vegetation ($F_{5,65} = 0.26$ and 0.42, $P = 0.611$ and 0.794, respectively), and the number of bacterial sequences was invariable across distance ($F_{5,59} = 1.33$, $P = 0.254$), but bare soil samples show lower sequence number compared with other vegetation treatments ($F_{5,59} = 3.15$, $P = 0.020$). After clustering at 97% sequence similarity, there were a total of 310 fungal and 4110 bacterial nonsingleton OTUs and 247 and 2972 singleton OTUs, respectively. Note that all singletons were omitted from the further analyses.

Estimation of soil microbial biomass by qPCR

Ribosomal copy numbers as a proxy for bacterial biomass estimated by qPCR (copy numbers per g soil dry weight) did not differ among the sampled rhizosphere environments. However, there was a near significant trend of increasing bacterial copy numbers with distance from the glacier terminus ($t = 1.96$, $P = 0.055$; Fig. 1). In contrast to bacteria, fungal copy numbers unequivocally increased with distance from the glacier terminus ($t = 2.33$, $P = 0.023$; Fig. 1), although the rates differed among the sampled vegetation; fungal biomass increased more slowly in *Abies*-associated soils than in the nonvegetated soils ($t = -2.12$, $P = 0.038$) and increased at a greater rate in *Luetkea*-associated soils than in the nonvegetated soil ($t = 3.98$, $P < 0.001$). Fungi to bacteria (F/B) biomass ratio were stable along this primary successional chronosequence, suggesting similar rates of fungal and bacterial biomasses accumulation (Fig. 1).

Fungal communities

Soil fungal communities were strongly dominated by diverse Ascomycota with 192 OTUs (61.9%) and 27 962 sequences (70.8%) followed by Basidiomycota with 84 OTUs (27.1%) and 3111 sequences (7.9%) (Fig. 2a,b). We also detected some Chytridiomycota with 10 OTUs (3.2%) and 66 sequences (0.2%), and Glomeromycota with 6 OTUs (1.9%) and 39 sequences (0.1%). We encountered an additional 18 OTUs (5.8%) comprised of 8331 sequences (21.1%) representing other basal fungi. Of these basal fungi, 13 OTUs showed best BLAST match to subphylum Mucoromycotina and 5 OTUs to subphylum Entomophthoromycotina. Of the 44 orders of Fungi, the most abundant were Hypocreales (9809 sequences, 24.8%) followed by Mortierellales (6740 sequences, 17.1%) and Helotiales (5620 sequences, 14.2%). On a family level, we observed 75 families, dominated by Mortierellaceae (6740 sequences, 17.1%), Cordycipitaceae (3750 sequences, 9.5%) and Clavicipitaceae (3553 sequences, 9.0%). Lastly, of the 154 genera classified, *Mortierella* (6740 sequences, 17.1%), *Trichocladium* (3042 sequences, 7.70%) and *Articulospora* (2944 sequences, 7.45%) were the most abundant.

The three most abundant OTUs were BLAST-assigned to *Mortierella alpina* (5858 sequences, Accession no. AJ878532.1, 93% query coverage, 99% max identity, family Mortierellaceae), *Cudoniella clavus* (3042 sequences, Accession no. DQ491502.1, 100% query coverage, 96% max identity, family Helotiaceae) and *Cordyceps bassiana* (2799 sequences, Accession no. EU673367.1, 100% query coverage, 100% max identity, family Cordycipitaceae). It is important to note that these described taxonomic abundances represent only sequences that we were able

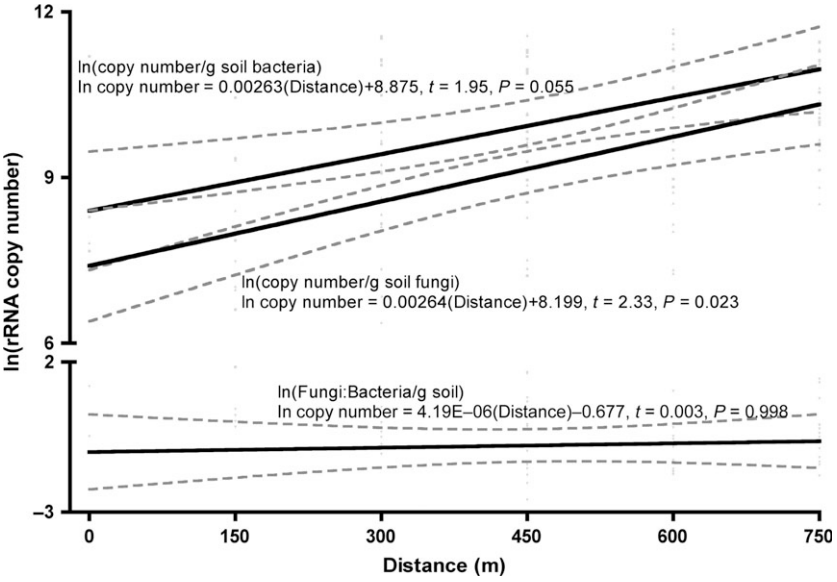

Fig. 1 Linear regression of the natural log of copy number per gram soil dry weight of bacteria, fungi and the fungi/bacteria across distance from Lyman glacier terminus. Biomass of fungi ($P = 0.023$) and bacteria ($P = 0.055$) increase with successional age, indicating increase in biomass as the vegetation establishes and substrate becomes more heterogeneous. The ratio of fungal to bacteria biomass remains stable ($P = 0.998$) across the chronosequence indicating similar rates of biomass accumulation.

to classify at high levels of taxonomic resolution through BLAST-n. There were many sequences that could not be classified (for example, 100 OTUs were not classified at the family level) and may thus represent novel OTUs.

Bacterial communities

Bacterial OTUs were distributed taxonomically as follows (Fig. 2c,d): the most abundant phyla were Proteobacteria with 1486 OTUs (36.2%) and 31 055 sequences (31.8%), followed by Actinobacteria with 612 OTUs (14.9%) and 18 995 sequences (19.4%) and Acidobacteria with 401 OTUs (9.8%) and 12 544 sequences (12.8%). Of particular note are the 68 OTUs representing the photosynthetic Cyanobacteria (1.7%) with 2712 sequences (2.8%). Of the 159 classified orders, Actinomycetales (11 415 sequences, 11.7%), Rhizobiales (7885 sequences, 8.1%) and Sphaerobacterales (6879 sequences, 7.0%) were the most abundant. Of the 198 OTUs classified to a family, those assigned to Sphaerobacteraceae (6879 sequences, 7.0%), Acetobacteraceae (5107, 5.2%) and Ktedonobacteraceae (4247, 4.4%) were the most abundant. Finally, of the 450 observed and taxonomically assigned genera, the most abundant were *Sphaerobacter* (6879 sequences, 7.0%), Gp1 (undefined Acidobacteria genus, 4419 sequences, 4.5%) and *Ktedonobacter* (4242 sequences, 4.3%). Many of the observed OTUs may represent novel taxa, as 1757 OTUs could not be assigned to a family and 1539 to a genus.

Microbial richness and diversity

Our rarefaction analyses suggest that although a large proportion of the fungal and bacterial richness was captured in our sampling, a greater sampling effort is needed for complete saturation (Fig. 3). Richness and diversity estimators (S, 1-D, E_D) showed distinct patterns that differed between fungal and bacterial communities (Fig. 4). Fungal OTU richness (4a), diversity (4b) and evenness (4c) estimators did not respond to distance from glacier terminus, plant association or their interaction (Table 1). In contrast, bacterial OTU richness (4d) increased over distance from glacier terminus ($F_{1,63} = 7.76$, $P = 0.007$) and differed between plant-associated environments ($F_{4,59} = 5.99$, $P < 0.001$). There was no significant interaction between the distance from the glacier terminus and the plant-associated environments, suggesting that these differences were additive and stable across the sampled glacier forefront. Although the complement of Simpson's diversity estimates (4e) for bacteria did not differ among the environments, bacterial community evenness (4f) decreased over distance from the glacier terminus ($F_{1,63} = 4.75$, $P = 0.034$). However, it did not differ among the plant-associated environments (Table 1).

Analyses of OTU distribution

Based on Bonferroni-corrected analyses testing whether OTUs are randomly distributed following a Poisson distribution across Lyman glacier forefront, a total of 240 bacterial OTUs (9.5% of all bacterial OTUs) deviated from the presumed Poisson distribution of randomness as expected if allochthonous propagule sources dominated the community assembly across the forefront. It is notable that nearly twice as great a proportion of fungal OTUs (59 or 18.79%) deviated from the Poisson distribution of randomness. These data strongly suggest that fungi – and to a lesser degree also bacteria – are

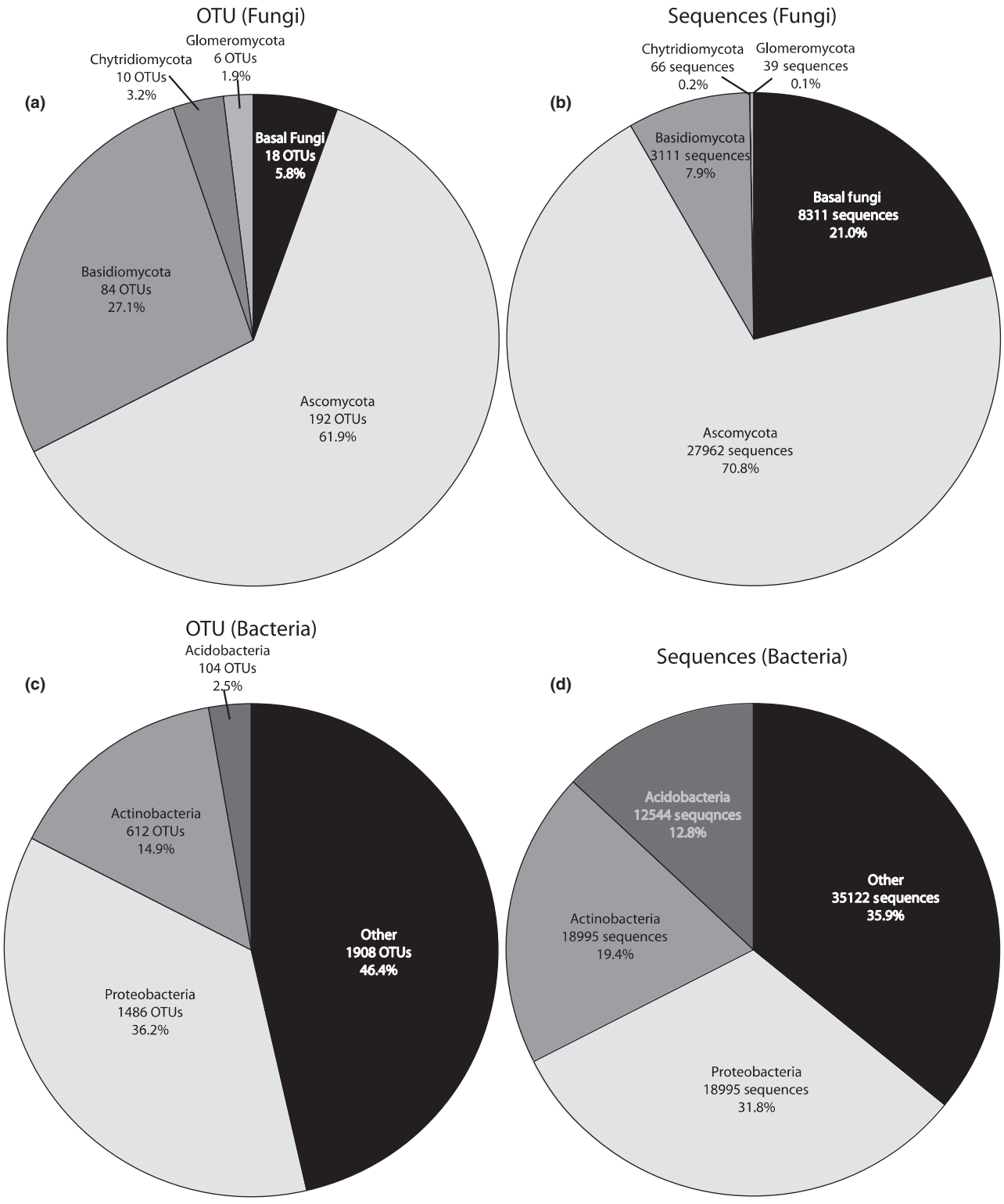

Fig. 2 Taxonomic distribution of fungal and bacterial OTUs and sequences on the Lyman glacier forefront. Fungal OTUs (a) are dominated by diverse Ascomycota and Basidiomycota. Fungal sequence counts (b) are dominated by Ascomycota and include a significant proportion of basal fungal lineages. Bacterial OTUs (c) and sequences (d) are dominated by Proteobacteria, Acidiobacteria and Acidiobacteria, but include large numbers of representatives from other phyla.

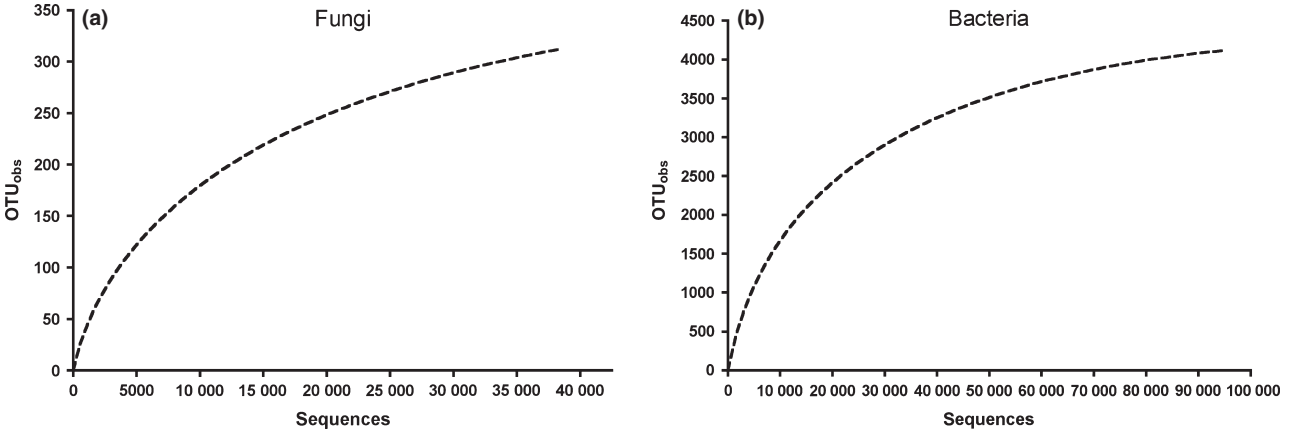

Fig. 3 Rarefaction analysis indicates that the fungal (a) and bacterial (b) communities approach saturation. Note the difference in axis scales.

nonrandomly distributed across this glacier forefront. Such nonrandom distributions suggest predictable community trajectories along the glacier forefront [Table S2 (Supporting Information) for Poisson test statistics] and/or effects of plant establishment.

Analyses of core taxa

Fungi. Core OTUs (present in ≥50% of samples) across the entire landscape or associated with different plants had affinities within subphyla Mucoromycotina and Pezizomycotina, two common and ubiquitous subphyla (see Table 2 for complete list of core taxa responding to distance and associated test statistics). By our definition, there were six core fungal OTUs across the entire data set. Of these, five changed in frequency over the distance from the glacier terminus (linear regression with Bonferroni correction), suggesting their correlation with changing community and ecosystem structure. Two of these core OTUs also responded to vegetation: OTU9 [*Cudoniella clavus*-saprobic] occurred less frequently in *Saxifraga* than in *Abies*; OTU104 [*Phialocephala sphaeroides* – a putative dark septate endophyte] occurred less frequently in *Saxifraga* and bare soil than in *Abies*). Core OTUs associated with *Abies* consisted of ten OTUs, one of which increased significantly in frequency with distance (OTU142 [*Serea difformis* – a putative saprobe]). *Luetkea* core consisted of twelve OTUs, six of which increased in abundance over distance from the glacier terminus [OTUs 3, 5, 13, 18, 24, 109 (*Pochonia bulbillosa* – insect parasite, *Lecythophora sp.* – saprobe, *Satchmopsis brasiliensis* - saprobe, *Articulospora tetracladia* – saprobe, *Cryptococcus skinneri* – wood pathogen, *Cladophialophora minutissima* – saprobe)]. *Phyllodoce* core consisted of eleven OTUs, none of which changed significantly with distance. *Saxifraga* core consisted of eight OTUs, one of which decreased in frequency across the forefront [OTU81 (*Penicillium citreonigrum* – saprobe)]. Nonvegetated soils had six core OTUs, two of which increased with distance from the glacier terminus [OTUs 1, 27 (*Mortierella alpina* – saprobe and *Mortierella elongata* – saprobe)].

Core taxon analyses focusing on the age of the substrate underlined the specificity in core membership with respect to distance from the glacier terminus. In all, 70 OTUs were considered as core OTUs in at least one of the distances – interestingly, 49 OTUs were core to only one distance. Of note is the abundance of an OTU (OTU 1) with affinity to *Mortierella alpina* (AJ878532.1). This OTU is a core taxon in all but samples collected adjacent to the glacier terminus. Remarkably, this OTU represents the most abundant sequence in all samples across the glacier forefront except those closest to the glacier where it was completely absent.

Bacteria. We identified 137 bacterial core OTUs that occurred in ≥50% of all samples. A total of twenty core OTUs (14.6%) changed in frequency (linear regression with Bonferroni correction): 18 increased and 2 decreased in frequency with distance from glacier terminus (Table 3). Overall, when the core OTUs were analysed for response to distance from the glacier terminus, the number of core bacterial OTUs increased ($F_{1,4} = 18.12$, $P = 0.013$).

Core OTUs from *Abies* samples consisted of 182 OTUs, but none of them changed in frequency with distance from the glacier terminus (Table 3). Similarly, *Phyllodoce* samples included 244 and *Saxifraga* samples 203 core OTUs, none of which changed in frequency over the chronosequence. *Luetkea* samples included 140 core OTUs with only two (1.4%) changing with distance from glacier, both of which increased. Nonvegetated soils consisted of 107 core OTUs, five of which (4.7%) increased in frequency over distance from glacier

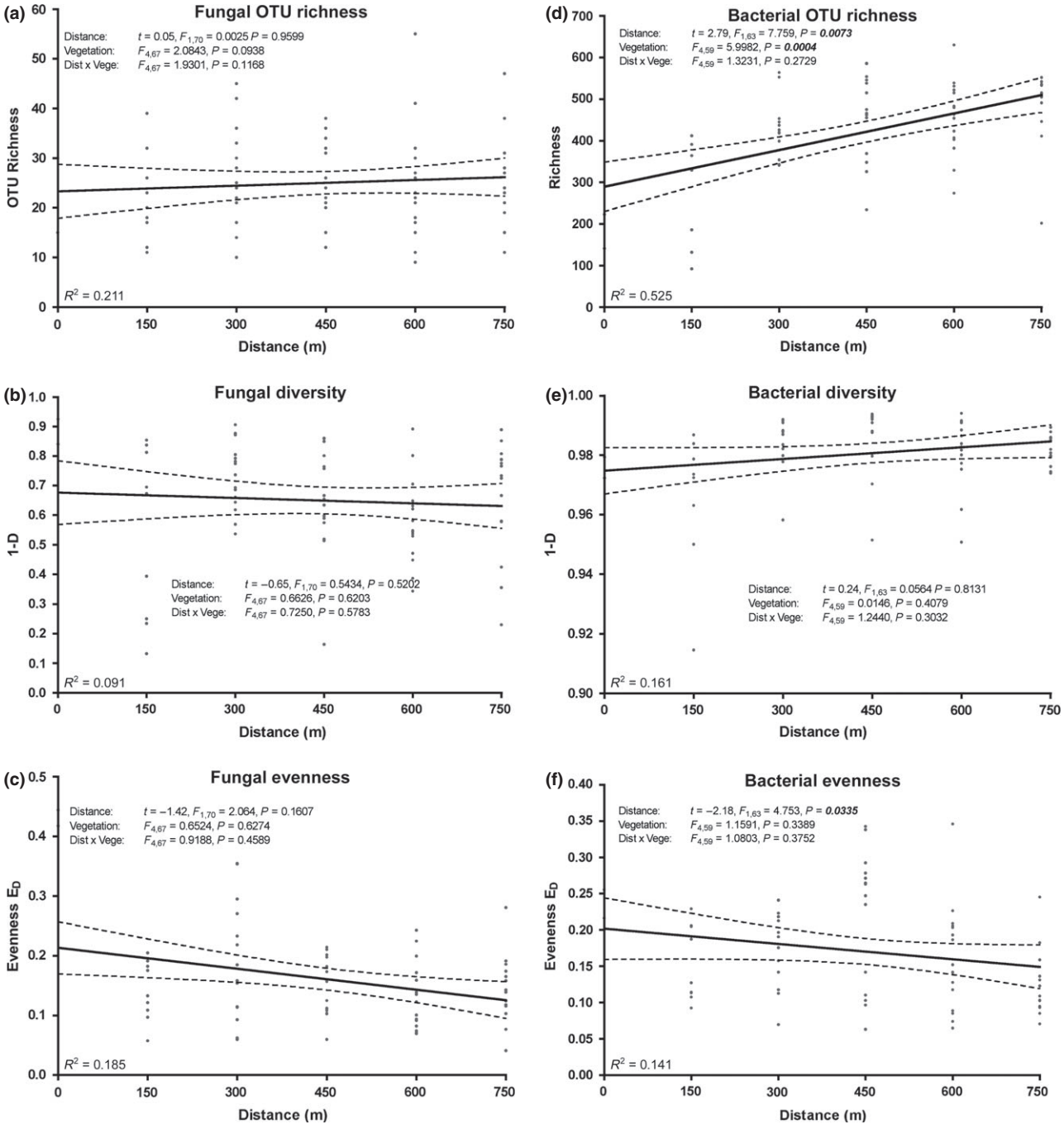

Fig. 4 Diversity indices for fungal and bacterial communities along Lyman Glacier chronosequence. Fungal community OTU richness (a), diversity [1-*D*] (b) and evenness (c) show stable and do not change with distance from glacier terminus (both linear regression and ANOVA). In contrast, bacterial communities differ in richness (d) across distance and vegetation. Bacterial diversity [1-*D*] (e) does not change with distance from glacier or with vegetation, whereas bacterial evenness (f) decreases with distance from the glacier terminus. We provide the *t*-statistics for the slope terms and *F*-statistics for distance for the complete model with distance as a continuous variable ($df = 1$) as well as *F*-statistics for vegetation and vegetation*distance interaction terms.

terminus, while none decreased. Of the 137 bacterial core OTUs across all treatments, 14 show a response to vegetation indicating some specific plant–bacterial associations. Of these 14 responding OTUs, nine differed in frequency between plant-associated and bare soils (Table 3). OTUs 1, 44, 53, 68, 119 and 203 were less frequent in bare than in plant-associated soils, whereas their frequencies did not differ among the

Table 1 Multiple linear regression analyses of OTU richness, diversity and evenness estimators across plant-associated soils. Non-vegetated Bare soil is used as a reference. Plant-associated intercept and slope estimates indicate difference in relation to Bare soil. OTU richness estimates are reported as raw OTU counts, whereas diversity and evenness are reported as arcsin [√(calculated values)]

Treatment	Intercept ± SE	Slope ± SE
OTU richness (fungi)		
Bare soil†	***25.42 ± 3.16******	$2.98 \times 10^{-4} \pm 5.91 \times 10^{-3}$
Abies	−0.94 ± 2.46	$1.66 \times 10^{-2} \pm 1.35 \times 10^{-2}$
Luetkea	2.85 ± 2.16	$\mathbf{-2.24 \times 10^{-2} \pm 1.08 \times 10^{-2}}$*
Phyllodoce	4.07 ± 2.66	$-1.22 \times 10^{-2} \pm 1.45 \times 10^{-2}$
Saxifraga	−0.96 ± 2.17	$1.70 \times 10^{-2} \pm 1.03 \times 10^{-2}$
OTU richness (bacteria)		
Bare soil†	***357.4 ± 34.4*****	***0.18 ± 0.06****
Abies	26.4 ± 26.4	−0.04 ± 0.15
Luetkea	11.3 ± 21.5	0.08 ± 0.1
Phyllodoce	***61.7 ± 29.5****	−0.31 ± 0.16
Saxifraga	10.9 ± 21.1	0.16 ± 0.1
Diversity (1-*D*, fungi)		
Bare soil†	***0.788 ± 0.088*****	$-1.20 \times 10^{-4} \pm 1.64 \times 10^{-4}$
Abies	−0.03 ± 0.069	$-9.75 \times 10^{-5} \pm 3.76 \times 10^{-4}$
Luetkea	0.077 ± 0.06	$-2.46 \times 10 -4 \pm 3.01 \times 10^{-4}$
Phyllodoce	0.015 ± 0.074	$8.13 \times 10^{-5} \pm 4.05 \times 10^{-4}$
Saxifraga	0.0037 ± 0.06	$4.15 \times 10^{-4} \pm 2.87 \times 10^{-4}$
Diversity (1-*D*, bacteria)		
Bare soil†	***1.44 ± 0.016*****	$-3.80 \times 10^{-6} \pm 2.94 \times 10^{-5}$
Abies	0.001 ± 0.012	$8.90 \times 10^{-8} \pm 7.18 \times 10^{-5}$
Luetkea	−0.002 ± 0.01	$2.76 \times 10^{-6} \pm 4.73 \times 10^{-5}$
Phyllodoce	0.03 ± 0.014	$-1.42 \times 10^{-4} \pm 7.45 \times 10^{-5}$
Saxifraga	−0.01 ± 0.009	$7.11 \times 10^{-5} \pm 4.82 \times 10^{-5}$
Evenness (E_D, Fungi)		
Bare soil†	***0.189 ± 0.028*****	$-7.48 \times 10^{-5} \pm 5.21 \times 10^{-5}$
Abies	−0.009 ± 0.022	$-3.06 \times 10^{-5} \pm 1.19 \times 10^{-4}$
Luetkea	0.005 ± 0.019	$8.67 \times 10^{-5} \pm 9.53 \times 10^{-5}$
Phyllodoce	−0.029 ± 0.023	$7.62 \times 10^{-5} \pm 1.28 \times 10^{-4}$
Saxifraga	0.0143 ± 0.019	$1.16 \times 10^{-6} \pm 9.09 \times 10^{-5}$
Evenness (E_D, bacteria)		
Bare soil†	***0.499 ± 0.038*****	$\mathbf{-1.58 \times 10^{-4} \pm 7.01 \times 10^{-5}}$*
Abies	−0.028 ± 0.029	$6.83 \times 10^{-5} \pm 1.71 \times 10^{-4}$
Luetkea	−0.024 ± 0.024	$8.01 \times 10^{-5} \pm 1.13 \times 10^{-4}$
Phyllodoce	0.058 ± 0.033	$-3.48 \times 10^{-4} \pm 1.78 \times 10^{-4}$
Saxifraga	−0.018 ± 0.023	$8.06 \times 10^{-5} \pm 1.15 \times 10^{-4}$

†Bare soil was selected as a reference level to emphasize the contrast between vegetation nonvegetated soils.
Significant values are in bold and italics and test the null hypotheses (H_0: intercept $_{Abies,\ Luetkea,\ Phyllodoce\ \text{or}\ Saxifraga}$ − intercept $_{\text{Ref Bare Soil}} = 0$; and H_0: slope $_{Abies,\ Luetkea,\ Phyllodoce\ \text{or}\ Saxifraga}$ − slope $_{\text{Ref Bare Soil}} = 0$). In other words, significant *P*-values here indicate that there is a difference between intercept or slope terms for treatments *Abies*, *Luetkea*, *Phyllodoce* or *Saxifraga* compared with bare soil. Level of significance indicated by asterisks, *refers to $0.01 < P \leq 0.05$, **refers to $0.001 < P \leq 0.01$, ***refers to $P \leq 0.001$.

plant-associated soils (OTUs 1, 53, 68 were placed with strong bootstrap support to the putatively nitrogen-fixing order Rhizobiales; OTUs 44, 119, 203 placed to order Xanthomonadales, and classes Gp6 and Gp3, respectively, with unknown function). In contrast, OTUs 60 (100% bootstrap support to likely photosynthetic Cyanobacteria), 75 (Gp1 with unknown function) and 486 (100% bootstrap support to phylum Chloroflexi [45% bootstrap support to genus *Sphaerobacter*], a putative aerobic thermophile adapted to the harsh UV-exposure characteristic of the bare soils) were more frequent in bare soil than plant-associated samples. These data suggest that plant establishment primarily controls core bacterial communities and their assembly. We emphasize the contrast between fungal and bacterial communities: while many bacteria responded to plant presence, the core fungal OTUs rarely responded to the presence or the taxon identity of the sampled plants, even though the plant species were specifically selected to represent differing root-symbioses with fungi.

Table 2 Core fungi (found in at least 50% of samples) that change in frequency with successional age (distance from the glacier terminus) using Bonferroni-corrected liner regression for combined vegetation sampling and different plant-associated soils. Direction of change indicates if the frequency of OTUs increased (↑) or decreased (↓) across distance from the glacier. Species affinity refers to the best BLASTn match (nr/nt) with the exclusion of uncultured/environmental samples

OTU	*t*-ratio	*P*-value	Direction of change	Species affinity	Phylum
All vegetation					
OTU1	2.0437	0.04479	↑	*Mortierella alpina*	'Zygomycota'
OTU13	−2.3303	0.02272	↓	*Satchmopsis brasiliensis*	Ascomycota
OTU27	2.8387	0.00594	↑	*Mortierella elongata*	'Zygomycota'
OTU3	5.4349	7.77E-07	↑	*Pochonia bulbillosa*	Ascomycota
OTU5	−4.0741	0.00012	↑	*Lecythophora sp.*	Ascomycota
OTU1	2.0437	0.04479	↑	*Mortierella alpina*	'Zygomycota'
Abies					
OTU142	2.3488	0.04071	↑	*Sarea difformis*	Ascomycota
Luetkea					
OTU109	−3.3111	0.00562	↑	*Cladophialophora minutissima*	Ascomycota
OTU13	−3.7416	0.00246	↑	*Satchmopsis brasiliensis*	Ascomycota
OTU18	−3.1925	0.00706	↑	*Articulospora tetracladia*	Ascomycota
OTU24	−3.2331	0.00653	↑	*Cryptococcus skinneri*	Basidiomycota
OTU3	3.4869	0.00401	↑	*Pochonia bulbillosa*	Ascomycota
OTU5	−3.4717	0.00413	↑	*Lecythophora sp.*	Ascomycota
Saxifraga					
OTU81	−3.2925	0.00583	↓	*Penicillium citreonigrum*	Ascomycota
Phyllodoce					
No significant frequency changes for core OTUs					
Bare soil					
OTU1	2.3021	0.03509	↑	*Mortierella alpina*	'Zygomycota'
OTU27	2.6516	0.01741	↑	*Mortierella elongata*	'Zygomycota'

Analyses of community composition

Fungi. While only few fungal OTUs seemed responsive to changes in environments along the successional chronosequence, analyses of NMS axes scores indicated strong and clear successional trajectories for fungal communities (Fig. 5a). Axis 1 (representing 23.2% of the variability) scores decreased drastically with increasing distance from glacier terminus ($t = -4.81$, $P < 0.001$). Treating distance from the glacier as a categorical variable, axis 1 scores differed across distance (ANOVA: $F_{1,69} = 5.59$, $P < 0.001$). This was mainly attributable to axis 1 scores in young substrates (at 0, 150 and 300 m distances from the glacier terminus) that were distinct from those in more developed substrates (at 450, 600 and 750 m from the glacier terminus; Tukey's HSD pairwise comparison at $\alpha = 0.05$). Axis 2 scores (representing 24.5% of the variability) also tended to increase ($t = 2.41$, $P = 0.019$) across distance from the glacier terminus. However, *Abies* communities were distinct from others, and their axis 2 scores decreased with increasing distance from the glacier terminus ($t = -2.22$, $P = 0.012$). These results suggest fungal community trajectories that are potentially distinct among establishing vegetation types during ecosystem development. Axis 3 (15.4% variation) showed neither a distinct trend across the distance along the glacier forefront nor any distinctions on communities across the distance from the glacier terminus as inferred from ANOVA.

In addition to the patterns with distance from glacier terminus, axes 1 and 2 scores also distinguished fungal communities in the establishing vegetation (ANOVA: $F_{9,61} = 5.59$, $P < 0.001$; $F_{9,61} = 3.20$, $P = 0.019$, respectively). Communities in *Luetkea* samples were distinct from bare and *Saxifraga* soils along axis 1 and from *Abies* along axis 2. Other soils remained indistinguishable based on our NMS analyses. These results suggest that this AM plant may strongly select fungal communities, whereas plants with other mycorrhizal habits do so to a lesser degree. Analyses of standard deviations of axis scores showed no change across distance ($F_{1,23} < 1.22$, $P > 0.2$ for all three axes) or vegetation ($F_{4,19} < 1.4$ $P > 0.3$ for all three axes) providing no support for fungal community convergence or divergence as a result of plant establishment or with distance from glacier terminus.

Bacteria. Soil bacterial communities showed strong trends with distance from the glacier terminus along all three NMS axes. Axis 1 (representing 19.1% of variation; $t = 3.67$, $P < 0.001$) and 3 (36.3% of variation; $t = 5.53$,

Table 3 Core bacteria (found in at least 50% of samples) that change in frequency with successional age (distance from the glacier terminus) using Bonferroni-corrected liner regression for combined vegetation sampling and different plant-associated soils. Direction of change indicates if the frequency of OTUs increased (↑) or decreased (↓) across distance from the glacier. Species affinity refers to the best Blastn match (nr/nt) with the exclusion of uncultured/environmental samples

OTU	*t*-ratio	*P*-value	Direction of change	Genus Affinity	Phylum
All vegetation					
OTU3	5.6094	4.84E-07	↑	*Sphaerobacter*	Chloroflexi
OTU11	5.7832	2.47E-07	↑	*Thermoflavimicrobium*	Firmicutes
OTU21	4.5428	2.57E-05	↑	*Gemmatimonas*	Gemmatimonadetes
OTU23	4.9367	6.14E-06	↑	*Humicoccus*	Actinobacteria
OTU25	5.4843	7.82E-07	↑	*Acidisphaera*	Proteobacteria
OTU45	5.6434	4.25E-07	↑	*Gemmata*	Planctomycetes
OTU63	−5.0748	3.68E-06	↓	*Caulobacter*	Proteobacteria
OTU71	3.9549	0.000196	↑	*Gp1*	Acidobacteria
OTU102	3.9867	0.000176	↑	*Rhodopseudomonas*	Proteobacteria
OTU105	3.8837	0.000249	↑	*Rhodopseudomonas*	Proteobacteria
OTU176	5.0846	3.55E-06	↑	*Gp2*	Acidobacteria
OTU215	5.8533	1.88E-07	↑	*Nitrospira*	Nitrospira
OTU300	−4.8361	8.89E-06	↓	*Solirubrobacter*	Actinobacteria
OTU327	3.8681	0.000262	↑	*Saxeibacter*	Actinobacteria
OTU382	4.7272	0.000013	↑	*Roseomonas*	Proteobacteria
OTU501	5.4177	1.01E-06	↑	*Ktedonobacter*	Bacteria_incertae_sedis
OTU508	4.6598	0.000016	↑	*Herbaspirillum*	Proteobacteria
OTU560	6.4705	1.66E-08	↑	*Gemmatimonas*	Gemmatimonadetes
OTU686	4.1925	0.000087	↑	*Flavisolibacter*	Bacteroidetes
OTU762	4.2865	6.34E-05	↑	*Zavarzinella*	Planctomycetes
Saxifraga					
No significant frequency changes for core OTUs					
Luetkea					
OTU538	5.3365	0.00013	↑	*Kozakia*	Proteobacteria
OTU572	5.6116	8.46E-05	↑	*Nitriliruptor*	Actinobacteria
Abies					
No significant frequency changes for core OTUs					
Phyllodoce					
No significant frequency changes for core OTUs					
Bare soil					
OTU16	5.8904	7.36E-05	↑	*Blastococcus*	Actinobacteria
OTU23	4.7931	0.000439	↑	*Humicoccus*	Actinobacteria
OTU35	5.5558	0.000125	↑	*Elioraea*	Proteobacteria
OTU215	4.8953	0.000369	↑	*Nitrospira*	Nitrospira
OTU1390	5.1931	0.000224	↑	*Conexibacter*	Actinobacteria

$P < 0.001$) scores increased with distance from glacier terminus (Fig. 5b), whereas axis 2 (representing 31.6% of variation) decreased with distance ($t = -3.58$, $P < 0.001$). Axes 2 and 3 also clearly distinguished the communities between the sampled plant species ($F_{9,55} = 3.46$, $P = 0.014$; $F_{9.55} = 12.60$, $P < 0.001$, respectively), whereas axis 1 scores did not differ among them. Axis 2 distinguished bacterial communities between *Phyllodoce* and nonvegetated soils, whereas axis 3 separated nonvegetated soils from all plant-associated soils (Fig. 5c) suggesting community filtering by vegetation regardless of the plants' mycorrhizal habits. Analyses of standard deviations for axes 1 and 2 provided no support for community convergence along the chronosequence. In contrast, standard deviations of axis 3 (36.3% of variation) decreased over distance from the glacier terminus ($t = -2.49$, $P = 0.021$) suggesting bacterial community convergence along axis 3 (Fig. 6), but this pattern vanished after accounting for different plant environments.

Discussion

We sequenced bacterial and fungal rRNA gene amplicons from a primary successional glacier forefront soils to analyse soil microbial community assembly along a deglaciated chronosequence. To our knowledge, this is the first study to address community assembly of bacteria and fungi simultaneously in a primary successional

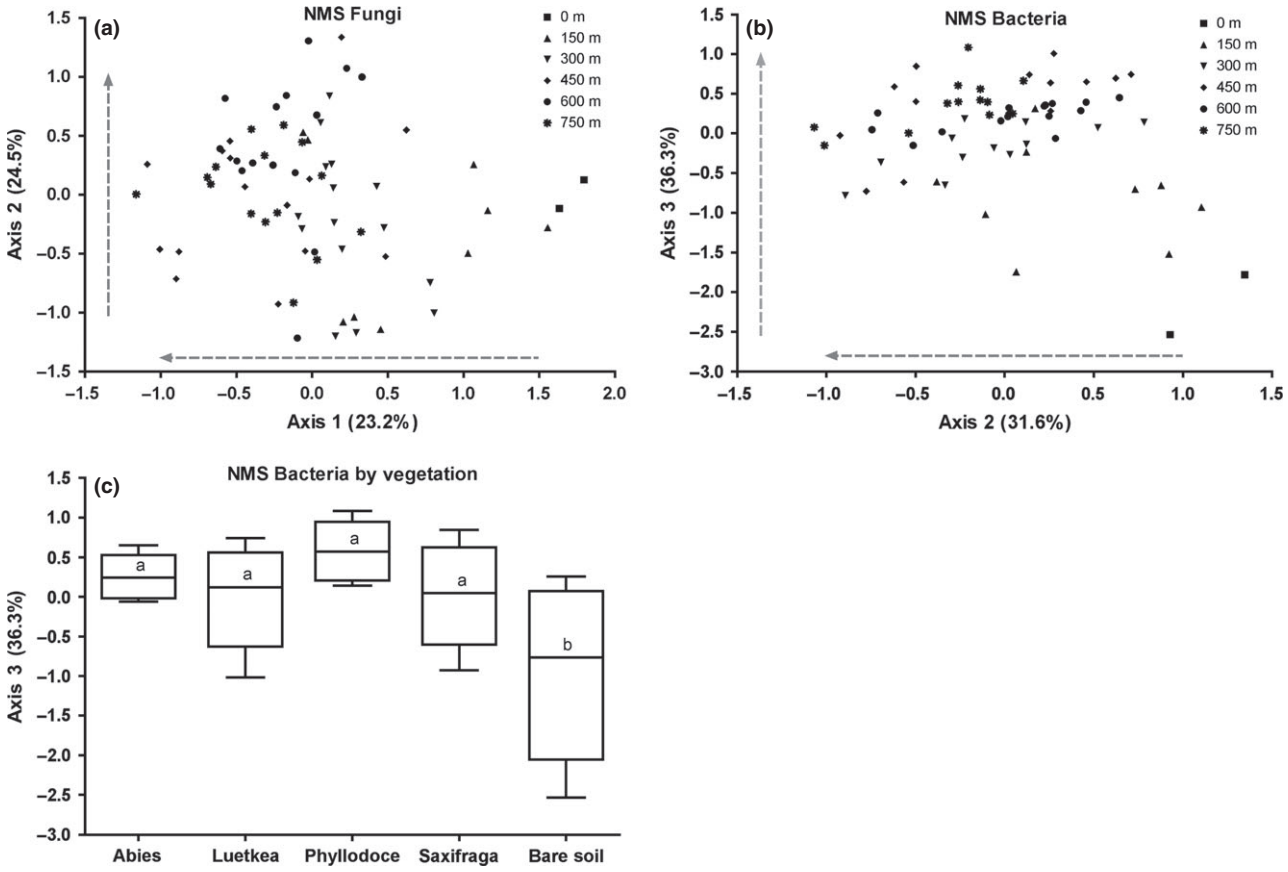

Fig. 5 Nonmetric Multidimensional Scaling (NMS) representation of fungal and bacterial communities along Lyman glacier chronosequence indicating community trajectories with increasing successional age. NMS of fungal (a) and bacterial (b) show strong successional trajectories with dashed arrows representing directionality of community shifts as indicated by significant linear regression statistics. Bacterial communities (c) in vegetated and bare soils are distinct along Axis 3 (different letters indicate significant differences in Tukey's HSD post hoc analysis at $\alpha < 0.05$).

system using high-throughput sequencing tools allowing for an unprecedented analysis of microbial community dynamics. Our analyses revealed three important and novel points about this early primary successional system that has continued to deglaciate for a century. *First*, the concurrent analyses of fungal and bacterial communities emphasize establishment and building organic legacies as a result of coinciding allochthonous resource arrival (see Hodkinson *et al.* 2001, 2002) as well as autochthonous microbial processes (see Kaštovská *et al.* 2007). *Second*, these analyses permitted comparisons between bacterial and fungal communities in early succession and identified differences either on the temporal scale or the trajectories between the two. *Third*, surprisingly and despite our choice of plants with differing mycorrhizal habits, we observed that bacterial communities are more strongly structured by established vegetation than fungal communities.

Our analyses highlighted diverse microbial communities in a successional system void of vegetation, corroborating conclusions of previous studies (Freeman *et al.* 2009a,b; Strauss *et al.* 2012; Zumsteg *et al.* 2012) and emphasizing the importance of microbial ecosystem functions in early primary succession to establish a pool of organic carbon and nitrogen in soil. Hodkinson *et al.* (2001, 2002) have argued that heterotrophs (particularly arthropods and arthropod-vectored inocula), not autotrophs, are the principal early drivers of primary successional dynamics. Both autochthonous (on site carbon and nitrogen accumulation) and allochthonous (arrival of organisms and debris from out of site sources) organic inputs probably contribute to the early establishment of ecosystem services. Three observations in our data support the importance of allochthonous inputs argued by Hodkinson *et al.* (2001, 2002). *First*, our data included five common fungal OTUs representing the entomopathogenic taxa in the subphylum Entomophthoromycotina. *Second*, the third most common fungal OTU (9.5% of all fungal sequences) was assigned to family Cordycipitaceae (*Cordyceps*) that includes a number of known entomopathogens. *Third*, OTU3 (with affinity to *Pochonia bulbillosa*, a known

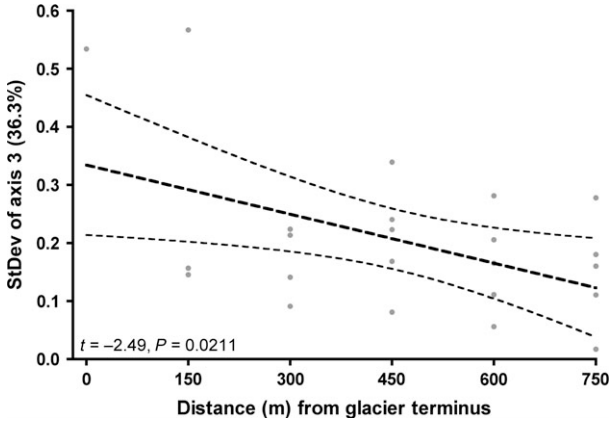

Fig. 6 Nonmetric multidimensional scaling (NMS) Axis 3 score standard deviations for bacterial communities along the Lyman Glacier chronosequence decrease with distance from the glacier terminus (successional age). Points represent standard deviations of vegetation treatments for each distance category. When the two points (0 and 150 m above 0.5) are removed from the analysis, the slope of the regression line no longer differs from zero. The analysis suggests that bacterial communities may converge with increasing successional age.

insect pathogen) was a core OTU across all samples and increased in frequency with successional age, suggesting a likely increase in insects colonized by this pathogen over the glacier chronosequence. In sum, our findings, combined with those of others, suggest that both autotrophic and heterotrophic microbial constituents play important roles in the accumulation of organic legacies in early successional systems.

In contrast to the commonly observed increase in plant community complexity and deterministic patterns of plant establishment during primary succession (del Moral *et al.* 1995; Jumpponen *et al.* 2012), fungal richness and diversity estimators were static across the Lyman glacier chronosequence. However, bacterial and plant communities seem to follow similar successional patterns characterized by increasing richness and declining evenness over successional time. These results suggest increasing heterogeneity in bacterial communities with increasing distance from glacier terminus. It is unclear whether plant establishment increases environmental heterogeneity leading to concomitant increases in bacterial community heterogeneity, or bacterial communities facilitate plant establishment by expediting pedogenesis. Our findings on the community dynamics are unlikely universal: for example, Blaalid *et al.* (2012) observed an increasing fungal OTU richness over successional time on a glacier forefront in Norway. Reasons for this incongruence are uncertain. However, it is important to bear in mind that our glacier forefront chronosequence represents less than a century of deglaciation, whereas that studied by Blaalid *et al.* (2012) spanned across centuries. While it is attractive to argue that fungal early successional trajectories are unique, the successional dynamics may be strongly influenced by regional and local factors. Furthermore, while fungal OTU richness and diversity estimates at our site were rather stable, fungal community structure and distribution were dynamic over the chronosequence – an observation congruent with other glacier systems (Blaalid *et al.* 2012).

Our analyses clearly show that distributions of many OTU are nonrandom. However, our analyses fell short in determining the drivers that structure these communities. The nonrandom distribution challenges the hypothesized random draw of microbial communities from an allochthonous propagule rain (Jumpponen 2003), possibly highlighting the difficulties of finding universalities, as multiple different processes may govern the OTU establishment. Our data suggest that some OTUs have distinct preferences for established vegetation or substrate age and that autochthonous propagation may be equally important in explaining the microbial establishment in successional landscapes. Moreover, compared with fungi, a smaller proportion of bacterial OTUs were nonrandom, suggesting that fungi probably have specific habitat requirements in early successional systems. However, our experimental design did not account for the primary drivers for this as indicated by lack of vegetation effect on the fungal community composition and the observed bacterial association with established vegetation. It is of particular note that many nonrandomly distributed OTUs were also core taxa (found in at least 50% of treatments) that exhibited shifts in frequency across the glacier forefront suggesting the potential importance of these core taxa on influencing patterns of spatial heterogeneity and community dynamics. Interestingly, of these nonrandomly distributed core fungal OTUs, all but one are putative saprobes and one was a potential insect pathogen. In addition, many core OTUs were unique to one distance category, which we interpret to suggest rapid turnover and dynamics in microbial communities during early succession. The number of core bacterial OTUs increased with successional age, which is suggestive of decreasing importance of stochastic allochthonous deposition in bacterial community establishment in this system. The core fungi and bacteria probably influence the community structure throughout the landscape and strongly contribute to overall differences in community dynamics over successional age.

Our NMS analyses clearly differentiated fungal and bacterial communities along the chronosequence. Additionally, bacteria were strongly differentiated among the plant-associated environments. Similarly to Trowbridge & Jumpponen (2004), we interpret these results

to indicate organismal niche preferences. Trowbridge & Jumpponen (2004) argued that it is indeed the successional age as well as the pedogenesis in the early successional environment that defines the fungal communities and selects the members that may successfully establish and survive. We extend this argument to account for bacterial successional trajectories. Unfortunately, our study – similarly to Trowbridge & Jumpponen (2004) – fails to provide adequately robust environmental data matrix to permit elucidation of those soil parameters that most strongly correlate with the observed communities. However, it is of note that previous studies in this system show that the soil organic matter, as well as carbon and nitrogen contents increase rather predictably as a result of increasing successional age and plant establishment (Cázares 1992; Jumpponen *et al*. 1998). As a result, the observed shifts in soil fungal and bacterial communities are probably correlated with accumulation of these organic legacies and the resultant shifts in soil chemical and physical properties.

One goal of our study was to evaluate microbial community divergence in a primary successional system similar to the dynamics observed in plant communities (del Moral 2009). We predicted that sampling plant hosts with distinct mycorrhizal habits would filter specific communities from the available propagule pool (Jumpponen & Egerton-Warburton 2005). However, despite the evident community turnover, our analyses did not support fungal community convergence. In contrast to the fungal communities, the presence of vegetation strongly influenced bacterial communities (with no observable difference in bacterial communities among different vegetation treatments) with bare soil bacterial communities being discrete from vegetation-associated communities. Taken together, this indicates a greater importance of stochastic processes in nonvegetated soils, *that is*, allochthonous propagule rain, whereas plant establishment partly drives deterministic processes in bacterial communities. Furthermore, in contrast to fungi, bacteria show evidence of community convergence as seen by the decreasing NMS axis 3 score standard deviations suggesting that communities may become similar to each other across the glacier forefront. We argue that this is evidence that early successional bacterial communities that are dictated by stochastic colonization with increasing determinism as a result of ecosystem development and/or pedogenesis.

Consistently with earlier studies, both fungal and bacterial biomasses (as proxied by qPCR assays) increased with distance along the Lyman glacier chronosequence (Ohtonen *et al*. 1999). Fungal and bacterial biomasses appeared to accumulate at similar rates as indicated by the stable F/B ratio. This is in contrast with previous work at this site reporting decreasing F/B ratio (Ohtonen *et al*. 1999). Those results were interpreted as a shift from bacteria- to fungus-dominated system coinciding with the vegetation establishment. The authors argued that as vegetation structure increases in complexity, fungi can more efficiently utilize available carbon sources compared with bacteria leading to the observed shift in biomass ratio. The reasons for this disparity remain unclear, but may include use of different biomass measurement tools as estimating biomass with qPCR may be influenced by many factors including variable copy numbers (Strickland & Rousk 2010) or the more than decade long time lag between the two studies.

Conclusions

Our study demonstrates that fungal and bacterial communities are dynamic along a primary successional chronosequence. Surprisingly, vegetation had a stronger effect on bacterial than fungal community dynamics even when the plant species were selected based on their mycorrhizal habit. Mycorrhizal habit should lead to a deterministic fungal community assembly, but our findings suggest other community-controlling mechanisms in early succession. More importantly, our data clearly indicated that microbial community dynamics are strongly influenced by distance along the Lyman glacier chronosequence and that these communities exhibit rapid turnover. While the communities overall may not differ among the sampled plants, the plant-associated microbial (fungal and bacterial) communities are enriched for certain community members. Furthermore, the core taxon frequency shifts with successional age suggest niche distinction and increasing importance of autochthonous inputs over the chronosequence. The bacterial and fungal communities differed in responses to establishing vegetation and exhibited dramatic differences in successional trajectories across the chronosequence. Taken together, our data highlight distinct successional dynamics between fungi and bacteria, but also provide insight into patterns that may be universal. Our findings warrant further investigation to pinpoint the primary drivers for observed similarities and differences.

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References

Ashkannejhad S, Horton TR (2006) Ectomycorrhizal ecology under primary succession on coastal sand dunes: interactions involving *Pinus contorta*, suilloid fungi and deer. *New Phytologist*, **169**, 345–354.

Baker GC, Smith JJ, Cowan DA (2003) Review and re-analysis of domain-specific 16S primers. *Journal of Microbiological Methods*, **55**, 541–555.

Barry RG (2006) The status of research on glaciers and global glacier recession: a review. *Progress in Physical Geography*, **30**, 285–306.

Blaalid R, Carlsen T, Kumar S *et al.* (2012) Changes in the root-associated fungal communities along a primary successional gradient analysed by 454 pyrosequencing. *Molecular Ecology*, **21**, 1897–1908.

Cázares E (1992) Mycorrhizal fungi and their relationship to plant succession in subalpine habitats. Ph.D Dissertation, Oregon State University, Corvallis, Oregon. 126 pp.

Cázares E, Trappe JM, Jumpponen A (2005) Mycorrhiza-plant colonization patterns on a subalpine glacier forefront as a model system of primary succession. *Mycorrhiza*, **15**, 405–416.

Chapin FS, Walker LR, Fastie CL, Sharman LC (1994) Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. *Ecological Monographs*, **64**, 149–175.

Colwell RK (2006) EstimateS: Statistical estimation of species richness and shared species from samples. Version 8.0.0. User's Guide and application. Available from: http://viceroy.eeb.uconn.edu/estimates/

Cramer W, Bondeau A, Woodward FI *et al.* (2001) Global response of terrestrial ecosystem structure and function to CO2 and climate change: results from six dynamic global vegetation models. *Global Change Biology*, **7**, 357–373.

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, **26**, 2460–2461.

Fierer N, Jackson JA, Vilgalys R, Jackson RB (2005) Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology*, **71**, 4117–4120.

Freeman KR, Martin AP, Karki D *et al.* (2009a) Evidence that chytrids dominate fungal communities in high-elevation soils. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 18315–18320.

Freeman KR, Pescador MY, Reed SC, Costello EK, Robeson MS, Schmidt SK (2009b) Soil CO_2 flux and photoautotrophic community composition in high-elevation, 'barren' soil. *Environmental Microbiology*, **11**, 674–686.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for Basidiomycetes – Application to the identification of mycorrhizae and rusts. *Molecular Ecology*, **2**, 113–118.

Helm DJ, Allen EB, Trappe JM (1996) Mycorrhizal chronosequence near Exit Glacier, Alaska. *Canadian Journal of Botony*, **74**, 1496–1506.

Hodkinson ID, Coulson SJ, Harrison J, Webb NR (2001) What a wonderful web they weave: spiders, nutrient capture and early ecosystem development in the high Arctic – some counter-intuitive ideas on community assembly. *Oikos*, **95**, 349–352.

Hodkinson ID, Webb NR, Coulson SJ (2002) Primary community assembly on land – the missing stages: why are the heterotrophic organisms always there first? *Journal of Ecology*, **90**, 569–577.

Jumpponen A (2003) Soil fungal community assembly in a primary successional glacier forefront ecosystem as inferred from rDNA sequence analyses. *New Phytologist*, **158**, 569–578.

Jumpponen A, Egerton-Warburton LM (2005) Mycorrhizal fungi in successional environments – a community assembly model incorporating host plant, environmental and biotic filters. In: *The Fungal Community* (eds Dighton J, White JF, Oudemans P), pp. 139–180. CRC Press, New York.

Jumpponen A, Mattson K, Trappe JM, Ohtonen R (1998) Effects of established willows on primary succession on Lyman Glacier forefront, North Cascade Range, Washington, USA: Evidence for simultaneous canopy inhibition and soil facilitation. *Arctic and Alpine Research*, **30**, 31–39.

Jumpponen A, Trappe JM, Cázares E (1999) Ectomycorrhizal fungi in Lyman Lake Basin: a comparison between primary and secondary successional sites. *Mycologia*, **91**, 575–582.

Jumpponen A, Trappe JM, Cázares E (2002) Occurrence of ectomycorrhizal fungi on the forefront of retreating Lyman Glacier (Washington, USA) in relation to time since deglaciation. *Mycorrhiza*, **12**, 43–49.

Jumpponen A, Jones KL, Mattox JD, Yaege C (2010) Massively parallel 454-sequencing of fungal communities in *Quercus* spp. ectomycorrhizas indicates seasonal dynamics in urban and rural sites. *Molecular Ecology*, **19**, 41–53.

Jumpponen A, Brown SP, Trappe JM, Cázares E, Strömmer R (2012) Twenty years of research on fungal-plant interactions on Lyman Glacier forefront - lessons learned and questions yet unanswered. *Fungal Ecology*, **5**, 430–442.

Kaštovská K, Stibal M, Šabacká M, Černá B, Šantrůčková H, Elster J (2007) Microbial community structure and ecology of subglacial sediments in two polythermal Svalbard glaciers characterized by epifluorescence microscopy and PLFA. *Polar Biology*, **30**, 227–287.

Knelman JE, Legg TM, O'Neill SP *et al.* (2012) Bacterial community structure and function change in association with colonizer plants during early primary succession in a glacier forefield. *Soil Biology & Biochemistry*, **46**, 172–180.

Kunin V, Hugenholtz P (2010) PyroTagger: a fast, accurate pipeline for analysis of rRNA amplicon pyrosequence data. *The Open Journal* **1**, 1–8.

Lane D (1991) 16s/23s rRNA sequencing. In: *Nucleic Acid Techniques in Bacterial Systematics* (eds Stackebrant E & Goodfellow M), pp. 115–175. John Wiley & Sons, West Sussex, United Kingdom.

McCune B, Mefford JM (1999) PC-ORD: Multivariate analysis of ecological data. Version 4. MjM Software, Gleneden Beach, Oregon, USA.

del Moral R (2009) Increasing deterministic control of primary succession on Mount St. Helens, Washington. *Journal of Vegetation Science*, **20**, 1145–1154.

del Moral R, Bliss LC (1993) Mechanisms of primary succession – insights resulting from the eruption of Mount St. Helens. *Advances in Ecological Research*, **24**, 1–66.

del Moral R, Titus JH, Cook AM (1995) Early primary succession on Mount St. Helens, Washington, USA. *Journal of Vegetation Science*, **6**, 107–120.

Mühlmann O, Peintner U (2008) Mycobionts of *Salix herbaceae* on a glacier forefront in the Austrian Alps. *Mycorrhiza*, **18**, 171–180.

Muyzer G, de Wall EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied Environmental Microbiology*, **59**, 695–700.

Nara K, Nakaya H, Wu BY, Zhou ZH, Hogetsu T (2003) Underground primary succession of Ectomycorrhizal fungi in a volcanic desert on Mount Fuji. *New Phytologist*, **159**, 734–756.

Oehl F, Schneider D, Sieverding E, Burga CA (2011) Succession of arbuscular mycorrhizal communities in the foreland of the retreating Morteratsch glacier in the Central Alps. *Pedobiologia*, **54**, 321–331.

Ohtonen R, Fritze H, Pennanen T, Jumpponen A, Trappe J (1999) Ecosystem properties and microbial community changes in primary succession on a glacier forefront. *Oecologia*, **119**, 239–246.

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acid Research*, **29**, e45.

Schmidt SK, Reed SC, Nemergut DR *et al.* (2008) The earliest stages of ecosystem succession in high-elevation (5000 meters above sea level), recently deglaciated soils. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 2793–2802.

Schütte UME, Abdo Z, Foster J *et al.* (2010) Bacterial diversity in a glacier foreland of the high Arctic. *Molecular Ecology*, **19**, 54–66.

Sigler WV, Zeyer J (2002) Microbial diversity and activity along the forefields of two receding glaciers. *Microbial Ecology*, **43**, 397–407.

Sikes BA, Maherali H, Klironomos JN (2012) Arbuscular mycorrhizal fungal communities change among three stages of primary sand dune succession but do not alter plant growth. *Oikos*, **121**, 1791–1800.

Strauss SL, Garcia-Pichel F, Day TA (2012) Soil microbial carbon and nitrogen transformations at a glacial foreland on Anvers Island, Antarctic Peninsula. *Polar Biology*, **35**, 1459–1471.

Strickland MS, Rousk J (2010) Considering fungal:bacterial dominance in soils – methods, controls, and ecosystems implications. *Soil Biology and Biochemistry*, **42**, 1385–1395.

Tedersoo L, Nilsson RH, Abarenkov K *et al.* (2010) 454 Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. *New Phytologist*, **188**, 291–301.

Tejesvi MV, Ruotsalainen AL, Markkola AM, Pirttila AM (2010) Root endophytes along a primary succession gradient in northern Finland. *Fungal Diversity*, **41**, 125–134.

Trowbridge J, Jumpponen A (2004) Fungal colonization of shrub willow roots at the forefront of a receding glacier. *Mycorrhiza*, **14**, 283–293.

Tscherko D, Hammesfahr U, Zeltner G, Kandeler E, Bocker R (2005) Plant succession and rhizosphere microbial communities in a recently deglaciated alpine terrain. *Basic and Applied Ecology*, **6**, 367–383.

Unterseher M, Jumpponen A, Öpik M *et al.* (2011) Species abundance distributions and richness estimations of fungal metagenomics – lessons learned from community ecology. *Molecular Ecology*, **20**, 275–285.

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, **172**, 4238–4246.

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, **73**, 5261–5267.

Zumsteg A, Luster J, Göransson H *et al.* (2012) Bacterial, archaeal, and fungal succession in the forefield of a receding glacier. *Microbial Ecology*, **63**, 552–564.

A.J. designed the experiment, collected samples and provided logistical support, S.P.B. performed all molecular work and data analyses; authors shared responsibilities for writing and editing the contribution.

Data accessibility

DNA Sequences: Sequence Read Archive: SRR943164-SRR943301; BioProject accession: PRJNA201483; BioSample accessions: SAMN02178414-SAMN02178482; SAMN 02265258.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 OTU count by sample matrices for fungal and bacterial OTUs.

Table S1 454 adaptor, primer, and multiplex tag (MIDs) sequences.

Table S2 Table of bacterial and fungal OTU test statistics testing each OTU for a fit to a Poisson distribution.