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Phylogenetic diversity analyses reveal disparity between fungal and bacterial communities during microbial primary succession

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ABSTRACT

Early community assembly of fungi and bacteria differ in many ways, including their contrasting successional trajectories. We examined if fungal and bacterial primary successional dynamics are phylogenetically constrained. Microbial communities were queried across a recently deglaciated forefront using three measures of phylogenetic diversity. We analyzed these data at the Kingdom (fungi) and Domain (bacteria) levels plus at less-inclusive taxonomic hierarchies (Phylum and Class) as well as selected, well-defined functional groups (N-fixing and photosynthetic bacteria). Fungi and bacteria differed in their phylogenetic distributions across successional age. Phylogenetic diversity estimates did not change over successional age for fungi, whereas bacteria were strongly structured phylogenetically over successional age. Further, our results suggest that analyses at Kingdom or Domain levels may prove inadequate to understand successional dynamics. Investigations should include both broad (Kingdom or Domain) and less inclusive groups (perhaps metabolically distinct taxa) to better dissect community dynamics.

1. Introduction

Primary successional dynamics of microbial communities are crucial for understanding community assembly rules, nutrient transformations and pedogenesis that may facilitate colonization and/or growth of vascular plants (Fierer et al., 2010). These early colonizing microorganisms drive patterns in ecosystem processes (Schmidt et al., 2008), transform/utilize ancient or recalcitrant carbon (Bardgett et al., 2007), and potentially facilitate the development of higher-trophic level food webs (Walker and de Moral, 2003; Jumpponen et al., 2015). During primary succession microbial communities establish onto virgin landscapes where little or no organic legacies exist, [*e.g.* volcanic substrata (Gemma and Koske, 1990), sand dunes (Brown, 1958), deglaciation (Schmidt et al., 2008), new island formation following to volcanic action (Marteinsson et al., 2015)]. Of these, studies on glacier forefronts can be particularly useful for examination of ecosystem development as many glaciers have well-documented recession rates and consist of short-term seres allowing for detailed examination through space for time substitution chronosequences (Walker et al., 2010). Molecular studies on microbial primary succession at glacier forefronts have been conducted via rRNA community fingerprinting (Sigler and Zeyer, 2002), cloning and sequencing (Jumpponen, 2003), PLFAs (Ohtonen et al., 1999; Tscherko et al., 2005), and T-RFLPs (Zumsteg et al., 2012). Studies on early microbial primary succession using next generation sequencing (NGS) have largely focused on shifting taxon abundances to understand community dynamics during succession (Schütte et al., 2010; Blaalid et al., 2012; Knelman et al., 2012) and often target only a fraction of the microbial constituents: *e.g.* root-associated fungi (Blaalid et al., 2012), or plant colonizing bacteria (Knelman et al., 2012). While targeting specific microbial community constituents allows for detailed interrogation of a particular successional aspect, focusing solely on one group (bacteria, fungi, or archaea) can limit our understanding of the microbial community as a whole.

Much about how fungal and bacterial communities assemble remains unresolved. Particularly, the similarity of successional dynamics of fungi and bacteria has been understudied. The first deep-sequencing (NGS) queries into joint fungal and bacterial primary

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successional dynamics are beginning to emerge (Brown and Jumpponen, 2014; Cutler et al., 2014). One intriguing result from these pioneering studies is that fungal and bacterial communities differ in their successional trajectories. Such findings are in contrast with earlier studies (Zumsteg et al., 2012) that demonstrate trajectory similarities possibly as a result of the limited depth of inquiry that T-RFLPs allow compared to NGS. Both Brown and Jumpponen (2014) and Cutler et al. (2014) found that bacteria and fungi have contrasting community wide and taxonomic successional trajectories but in different ways. Brown and Jumpponen focused on early successional dynamics (0–80 years) across a glacier forefront and found a tight link between plant establishment and bacterial communities, although both fungal and bacterial communities were mainly influenced by successional age. In contrast, Cutler and coauthors queried a more extensive successional timeframe (165–852 years) and found that fungi were closely linked to plant establishment but bacteria less so. The reason for this discrepancy is uncertain but may be a result of differing successional ages, substrate associated nutrient limitations (glacier forefront vs. volcanic deposition), or geographic location.

Colonization of newly developed substrates presents challenges as opportunities to colonize are hindered by strong environmental/biotic filtering (Jumpponen et al., 2015). For a glacier forefront, abiotic filters include strong UV irradiation, drastic diurnal temperature fluctuations, long-lasting snow cover, and extreme nutrient limitations (Jumpponen et al., 2012). Establishment is likely related to evolutionary histories that constrain the traits mandatory for successful establishment and dispersal (Dini-Andreote et al., 2015). That is, microbes that are suited for a given environment likely share similar niches through shared evolutionary histories that account for the success in establishment (Philippot et al., 2010). Such a phylogenetic signal that influences establishment may be even more evident for fungi (Maherali and Klironomos, 2007). It is in this light that cross-domain investigation into phylogenetic and evolutionary constraints of microbial succession can shed light into community assembly rules and successional dynamics.

Phylogenetic diversity (integration of phylogenetic distances into community ecology to infer community and functional dynamics; see Webb, 2000) frameworks have been widely applied to understand many communities (see Table 1 in Vamosi et al., 2009), including trees (Webb, 2000) and mammals (Cardillo, 2011). Despite the common use of phylogenetically conserved gene regions for bacterial and fungal amplicon libraries, studies of bacterial (Horner-Devine and Bohannan, 2006) and fungal (Anderson et al., 2004; Merckx et al., 2012; Rämä et al., 2014) phylogenetic diversity are rare and largely focus on specific groups of microbes at lower taxonomic levels. Rarer still are studies targeting microbial phylogenetic diversity across environmental gradients but some have focused on altitudinal effects (Bryant et al., 2008). Some bacterial phyla may shift phylogenetically with altitude (Wang et al., 2012), whereas no such shifts were apparent for Sebacinoid fungi (Garnica et al., 2012). To our knowledge, concurrent phylogenetic diversity analyses of fungi and bacteria are currently lacking. Here, we integrate evolutionary history of microbial communities assembling in a primary successional environment – a component crucial to an improved understanding of successional dynamics (O'Dwyer et al., 2012).

Bacterial communities may converge compositionally along glacier forefronts during early primary succession as evidenced by declining standard deviations of ordination loading scores with distance from the receding glacier's terminus (Brown and Jumpponen, 2014). Similarly, fungal communities may converge phylogenetically (Jumpponen et al., 2012): older communities are more phylogentically related as compared to more recently established communities. Evidence for bacterial and fungal convergence across early successional age is circumstantial at best (based on Jumpponen et al., 2012; Brown and Jumpponen, 2014) and the underlying reasons for these observations remain poorly understood. In this contribution, we explicitly test if the observed compositional convergence is phylogenetically constrained. Additionally, we employ a taxonomically hierarchical approach in our analyses and posit that the use of multiple taxonomic hierarchical ranks is required for a more complete understanding of microbial community assembly.

Table 1
Proportion of samples that are significantly phylogenetically clustered compared in 1000 iterations against randomly generated trees (null model – independent swap). NTI – Nearest Taxon Index and NRI – Net Relatedness Index tested using a 2 × 2 contingency table with Fisher's Exact Test. Non-significant P-values are presented parenthetically.

	NTI (significant experimental units)	NRI (significant experimental units)	Fisher's Exact Test P-value
All fungi	67.3%	44.2%	0.0015
Ascomycota	51.0%	57.1%	NS (0.685)
Basidiomycota	100%	100%	NS (1.00)
All Bacteria	57.1%	6.3%	<0.0001
Acidobacteria	66.7%	33.3%	0.0005
Actinobacteria	51.6%	12.9%	<0.0001
α-Proteobacteria	62.3%	0.0%	<0.0001
β-Proteobacteria	100%	40.0%	NS (0.167)
γ-Proteobacteria	100%	80.0%	NS (1.00)
Diazotrophs	60.0%	20.0%	NS (0.524)
Photosynthetic	40.0%	60.0%	NS (1.00)

2. Materials and methods

2.1. Sampling location

The forefield of Lyman Glacier is located within the Wenatchee National Forest in Glacier Peaks Wilderness Area in Washington State, USA (48°10′14″N, 120°53′44″W; ~1900 m a.s.l). The glacier has receded more than 1 km over the past ~120 years (Jumpponen et al., 1998). Plant (Jumpponen et al., 2012) successional dynamics as well as bacterial and fungal community succession in response to plant establishment (Brown and Jumpponen, 2014) have been previously characterized at this site. Soils at the forefront mainly consist of Entisols and are rocky and with limited nitrogen (less than 0.1% total soil N) and carbon (*ca* 3% total soil carbon by dry weight) under established vegetation near the terminal moraine (Jumpponen et al., 1998) with even more limited availabilities in unvegetated soils. There is no apparent increase in total N or C with forefront development in either vegetated or unvegetated soils. However, the forefront contains many surface depressions that may act as safe sites aiding plant establishment and viability (Jumpponen et al., 1999).

2.2. Sampling and sequence generation

We used soil samples and extracted DNA from previous research efforts (Brown and Jumpponen, 2014) in the current analyses. Briefly, topsoil was sampled along the deglaciated chronosequence (150 m, 300 m, 450 m, 600 m, and 750 m from the glacier terminus representing *circa* 90 years of successional time) for a total of 68 experimental units (across the five distances) and non-vegetated soil as well as rhizospheric soils from four plants with differing mycorrhizal habits were sampled. In our previous analyses (Brown and Jumpponen, 2014) successional age was much more influential in structuring microbial communities than vegetation (Brown and

Jumpponen, 2014). Consequently, we did not consider the vegetation in our present analyses. Previously, 454-amplicon libraries were generated for bacteria (primers B-9F and A-MID-541R where A is the sequencing adaptor, MID is 8-basepair unique molecular identifier tag, and B is the emPCR bead adhering adaptor) and fungi (primers A-MID-ITS1f and B-ITS4) (see Brown and Jumpponen, 2014 for further details). For this study, we reanalyzed the existing bacterial dataset and generated a new fungal library using primers that amplify the 5′ end of the Large Subunit (LSU) of the ribosomal RNA gene repeat (our previous ITS based analyses are not suitable for multiple sequence alignments required for phylogenetic diversity analyses).

LSU amplicons were generated using three technical replicates utilizing a two-step PCR to minimize MID-induced PCR biases (Berry et al., 2011) in 25 μL reaction volumes. Primary PCR conditions were: 1 μM forward and reverse primers (LR0R and LR3; *see* Amend et al., 2010), 5 ng template DNA, 200 μM of each deoxynucleotide (dNTPs), 2.5 mM $MgCl_2$, 5 μL 5x Green GoTaq® Flexi Buffer (Promega, Madison, WI), 7.8 μL molecular biology grade water, and 1 U GoTaq® Hot Start Polymerase (Promega, Madison, WI). Primary PCR parameters consisted of 94° initial denaturing for 4 min, 25 cycles of 94° denaturing for 1 min, 53° annealing for 45 s and 72° extension for 2 min followed by a 72° terminal extension for 8 min. Resultant PCR product was used as a template DNA for the secondary PCR (with primer fusion constructs that included 8-bp multiplexing tags (MID) and 454-sequencing specific linkers). Conditions of secondary PCR were: 5 μL primary PCR product, 0.5 μM forward and reverse primers (A-MID-LR0R and B-LR3 respectively), 2.5 mM $MgCl_2$, 200 μM of each deoxynucleotide, 5 μL 5x Green GoTaq® Flexi Buffer (Promega, Madison, WI), 7.3 μL molecular biology grade water and 1 U GoTaq® Hot Start Polymerase with PCR parameters of 94° initial denaturing for 4 min, 5 cycles of 94° denaturing for 1 min, 54° annealing for 1 min and 72° extension for 2 min followed by a 72° terminal extension for 10 min. Positive and negative controls were included and the latter remained free of contamination. The three technical replicates for each experimental unit were pooled and cleaned using Agencourt® AmPure® cleanup kit using a SPRIplate 96-ring magnet (Beckman Coulter, Beverly, MA). Resultant amplicons were pooled equimolarly and cleaned again with AmPure® and 454-pyrosequenced (GS FLX-Titanium, Roche Applied Science, Indianapolis, IN) at the Integrated Genomics Center at Kansas State University (Manhattan, KS).

2.3. Bioinformatic processing and analyses

The obtained bacterial and fungal sequences (individual fastq files for each experimental unit are in the Sequence Read Archive at NCBI as BioProject PRJNA201483) were processed in MOTHUR (v. 1.31.2; Schloss et al., 2009). The 454 flowgrams were denoised (PyroNoise; Quice et al., 2009) and the passing sequences aligned against the SILVA 16S reference (bacteria) or modified (see Brown et al., 2014) LSU alignment (James et al., 2006). Putative chimeras were identified (UCHIME; Edgar et al., 2011) and culled. Operational taxonomic units (OTUs) were demarcated at 97% similarity (UPGMA), singleton OTUs (Tedersoo et al., 2010; Brown et al., 2015) removed, and subsampled to equal depth per experimental unit (600 bacterial and 200 fungal sequences per experimental unit; see Gihring et al., 2012). OTUs were classified with the Naïve Bayesian Classifier (Wang et al., 2007) against Ribosomal Database Project's reference (16S v.9 or 28S v.7 rRNA reference) and non-target OTUs were removed. Where possible, OTUs were manually assigned to ecological roles based on genus level affinities. Using these classifier-based identities, a comprehensive list of sequence identifiers for fungi and bacteria, for the most abundant phyla (Basidiomycota, Ascomycota, Acidobacteria, and Actinobacteria) or classes (α-, β-, and γ-Proteobacteria) were generated. Remaining phyla were either too rare, too heterogeneous in their abundance across the landscape, or dominated by only few members that could confound phylogenetic diversity analyses. Additionally, sequence lists of functional groups – diazotrophs (genera with only nitrogen fixing members) and photosynthetic bacteria (genera with only photosynthesizing bacteria) – were generated based on these annotations. We also targeted mycorrhizal and entomopathogenic fungi but they were too skewed in abundance and thus omitted.

These lists were used to harvest sequences from the original sff files and new fasta and groups files generated containing only quality-verified sequences of the targeted groups (phyla, class, or functional group). These new fasta files were aligned as above to ensure the presence of only informative gaps. For each queried group, sequences were subsampled to equal number per experimental unit. Where subsampling lead to elimination of ≥25% of experimental units at a depth of 100 sequences per experimental unit, the experimental units were collapsed into one per distance from the glacier terminus (150 m, 300 m, 450 m, 600 m, 750 m) resulting of 5 functional experimental units. This lead to the following subsampling scheme: all Fungi—150 sequences per experimental unit, Ascomycota—150 sequences, Basidiomycota—700 sequences (collapsed samples), all Bacteria—300 sequences, Acidobacteria—125 sequences, Actinobacteria—150 sequences, α-Proteobacteria—100 sequences, β-Proteobacteria—850 sequences (collapsed), γ-Proteobacteria—750 sequences (collapsed), diazotrophs—70 sequences (collapsed), and photosynthetic Bacteria—46 sequences (collapsed). Relaxed Neighbor-Joining trees were generated for each subsampled and aligned fasta file using CLEARCUT (v.1.0.9; Sheneman et al., 2006) as embedded in MOTHUR.

Indices of phylogenetic diversity were calculated in R (v. 2.10.1; R Development Core Team 2007) using package *Picante* (V. 1.1-1; Kembel et al., 2010). Nearest Taxon Index (NTI) and Net Relatedness Index (NRI) (Webb, 2000) were calculated using the null model 'independent swap' (Gotelli and Entsminger, 2003) with 999 randomization runs with 1000 iterations; Faith's index of phylogenetic diversity (FI; Faith, 1992) was non-iterative. NTI is a measure of phylogenetic clustering of terminal nodes calculated as the average branch length between a sequence and its closest relative standardized by the maximum possible values for the tree. Thus, NTI is more sensitive to terminal proximity topological branching, whereas NRI is based on mean pairwise distance of the terminal nodes across the whole tree and thus more sensitive to deeper topological branching. Higher values of NTI and NRI indicate stronger phylogenetic clustering (compared to random trees) whereas lower values represent no phylogenetic clustering or overdispersion within communities. FI is the summative branch length between all members in a sample and correlates positively with taxon richness (Vamosi et al., 2009). We considered communities phylogenetically clustered (NTI and NRI) if observed phylogeny was more extreme than 1000 Monte Carlo randomized trees.

Changes in phylogenetic diversity (NRI, NTI or FI) over successional age were tested using regression analyses. As reported previously, vegetation had minimal effects on bacterial (bare soil was distinct from vegetated soils but soils under different plants were similar) or fungal succession, while both were strongly structured by successional age (Brown and Jumpponen, 2014). As a result, only distance from the glacier terminus (age of substrate exposure) was used here. Phylogenetic diversity estimates were regressed against distance from the glacier terminus using linear models whereby an increase in phylogenetic diversity evidences phylogenetic divergence, i.e., more established communities consist of more distant

community members, possibly due to an increase in niche diversity with ecosystem development. Conversely, decreasing phylogenetic diversity values evidences phylogenetic convergence suggestive of initial stochastic membership that is filtered such that later communities include members that are closely related. The obtained linear models tested for Lack of Fit and, where significant at $\alpha = 0.10$, further quadratic models were explored. If the quadratic models fit the data better (based on R^2 values and Lack of Fit tests), they are reported. For the collapsed samples, polynomial models were used only if they were superior in their explanatory power (based on Adjusted R^2 values). All regression analyses were conducted in JMP® (v. 7.0.2; SAS Institute, Cary, NC, USA).

3. Results

In all analyses, early successional communities showed evidence of phylogenetic clustering to some degree. However, the strength and clustering patterns differed between fungi and bacteria. Many fungal samples were consistently clustered when compared to random trees. Approximately half of the experimental units were significantly clustered in analyses that included fungi or only Ascomycota (Table 1), whereas all basidiomycete communities (collapsed into five samples – 150 m, 300 m, 450 m, 600 m, and 750 m) were clustered. Furthermore, the proportion of fungal experimental units that were significantly phylogenetically clustered for NTI and NRI differed (NTI > NRI; Fisher's Exact Test $P = 0.0015$). Similarly to the analyses focusing on fungi, bacteria in many samples were clustered phylogenetically and the frequency of clustered samples based on NTI was greater than that based on NRI ($P < 0.0001$; see Table 1). This disparity between NTI clustering and NRI clustering was sometimes drastic; more than half of the queried α-Proteobacteria samples were clustered based on NTI, whereas none appeared clustered using NRI.

Regression analyses of distance from the glacier terminus and phylogenetic diversity provide some interesting and contrasting results between fungi and bacteria. When phylogenetic diversity metrics for all fungi were regressed, the slope estimates for NTI ($t = 0.00$, $P = 0.999$; Fig. 1a), NRI ($t = 0.58$, $P = 0.566$; Fig. 1b), or FI ($t = -0.19$, $P = 0.859$; Fig. 1c) did not differ from zero. This is in contrast with the bacterial analyses: both NTI and NRI declined with distance from the glacier, but NTI more so than NRI ($t = -5.37$, $P < 0.0001$, see Fig. 1d; $t = -1.84$, $P = 0.0712$, Fig. 1e; respectively). This may suggest that for bacteria, niche diversity increases as the forefront develops. Further, the bacterial analyses indicated strong increases in phylogenetic branch length (FI; $t = 5.93$, $P < 0.0001$; Fig. 1f) with distance from the glacier, possibly indicating inclusion of more distantly related taxa with successional age.

In the analyses of the lower (less inclusive) fungal taxonomic groups, a slightly different story emerged. Regression analyses focusing on Basidiomycota indicated no relationship with successional age for NTI ($t = -0.81$, $P = 0.479$; Fig. 2a), NRI ($t = -0.62$, $P = 0.578$; Fig. 2b) or FI ($t = 0.77$, $P = 0.497$; Fig. 2c). Similar analyses of Ascomycota indicated that regression models that best fit the NTI data were unimodal with a peak between 450 m and 600 m from the glacier terminus ($df = 48$, $P = 0.0096$; Fig. 2d) whereas NRI and FI were unchanging ($t = 0.79$, $P = 0.435$; Fig. 2e and $t = -1.28$, $P = 0.207$; Fig. 2f – respectively).

The different taxonomic levels and functional groups of bacteria showed some intriguing differences. Acidobacteria followed the results of the combined bacterial groups: NTI ($t = -7.50$, $P < 0.0001$; Fig. 3a) and NRI ($t = -7.45$, $P < 0.0001$; Fig. 3b) strongly and highly significantly decreased with distance from the glacier terminus, whereas FI ($t = 8.65$, $P < 0.0001$; Fig. 3c) increased. Actinobacteria results were slightly different: similarly to Acidobacteria, NTI decreased ($t = -4.10$, $P = 0.0001$; Fig. 3d) and FI increased ($t = 2.60$, $P = 0.0116$; Fig. 3f) with distance, but in contrast, NRI did not change ($t = -1.30$, $P = 0.1994$; Fig. 3e). These Actinobacterial data appear increasingly heteroskedastic with distance from the glacier terminus, although regression analyses of the absolute value of residuals indicate that the apparent increase in variance did not preclude analysis ($t = 1.19$, $P = 0.2394$). Even within the Proteobacteria, there were clear contrasts. The NTI estimates for α-Proteobacteria decreased ($t = -3.28$, $P = 0.0019$; Fig. 3g) with distance, whereas NRI remained stable ($t = -0.38$, $P = 0.7025$; Fig. 3h) and FI increased ($t = 3.77$, $P = 0.0004$; Fig. 3i). The β-Proteobacteria analyses suggested that for both NTI and NRI, quadratic models were the most appropriate (NTI – $df = 4$, $P = 0.0475$, Fig. 3j; NRI – $df = 4$, $P = 0.0160$, Fig. 3k) responding strongly to distance from the glacier terminus but in opposite directions. The best-fit for NTI was concave quadratic model with a local minimum around 600 m, whereas the best-fit for NRI was convex quadratic model with a maximum around 450 m from the glacier terminus. However, total branch length (FI) increased linearly with distance ($t = 4.80$, $P = 0.0172$; Fig. 3l) suggesting an increase in β-Proteobacterial richness. The γ-Proteobacterial NTI was best explained with quadratic models and responded to distance from the glacier terminus ($df = 4$, $P = 0.0079$; Fig. 3m) with a minimum around 550 m, whereas there were no good linear or quadratic models for NRI ($df = 4$, $P = 0.1291$; Fig. 3n). The γ-Proteobacterial FI followed a convex quadratic regression with distance from the glacier ($df = 4$, $P = 0.0105$; Fig. 3o) with a maximum around 550 m. Finally, the bacterial functional groups were rarely responsive to distance from the glacier. The diazotroph communities were non-responsive for NTI ($t = -1.03$, $P = 0.3779$; Fig. 3p), NRI ($t = 0.81$, $P = 0.4763$; Fig. 3q) or FI ($t = 0.12$, $P = 0.9120$; Fig. 3r). In contrast, NTI ($t = -0.46$, $P = 0.6777$; Fig. 3s) and NRI ($t = 0.03$, $P = 0.9771$; Fig. 3t) estimates of the photosynthetic bacteria were unresponsive, but FI estimates responded positively ($t = 3.49$, $P = 0.0396$; Fig. 3u) to distance from the glacier terminus indicative of an increase on carbon fixing bacterial richness that establish independent of phylogenetic constraints.

4. Discussion

The establishment and early community assembly of fungi and bacteria in primary successional systems share many similarities including the stochastic arrival of allochthonous propagules onto virgin substrates. The propagating species can either establish or fail to initiate metabolic activity, largely driven by environmental and physiological restrictions (Jumpponen and Egerton-Warburton, 2005). It has previously been demonstrated that many microbes are active (or potentially active) in recently deglaciated soils (Knelman et al., 2014) and able to quickly utilize available nutrients or aerially deposited carbon rich substrates (*e.g.* pollen). These burgeoning communities are further filtered via biotic interactions and priority effects can control the success of competing species with similar niche requirements (Peay et al., 2012). Our results indicate that one should not consider the assembly of highly inclusive groups (Kingdom or Domain) as homogeneous and uniform. Rather, to understand assembly dynamics, lower and less inclusive groups must be considered. Considering taxonomic subgroups, functional groups, or perhaps even individual species allows for a deeper and more complete understanding of the microbial community assembly.

In our previous characterization of microbial succession at this site (Brown and Jumpponen, 2014), fungal and bacterial communities follow contrasting successional trajectories. This study further establishes that fungi and bacteria, as well as groups within them, differ in their successional dynamics. Phylogenetic diversity estimates of the fungal communities were largely unresponsive to

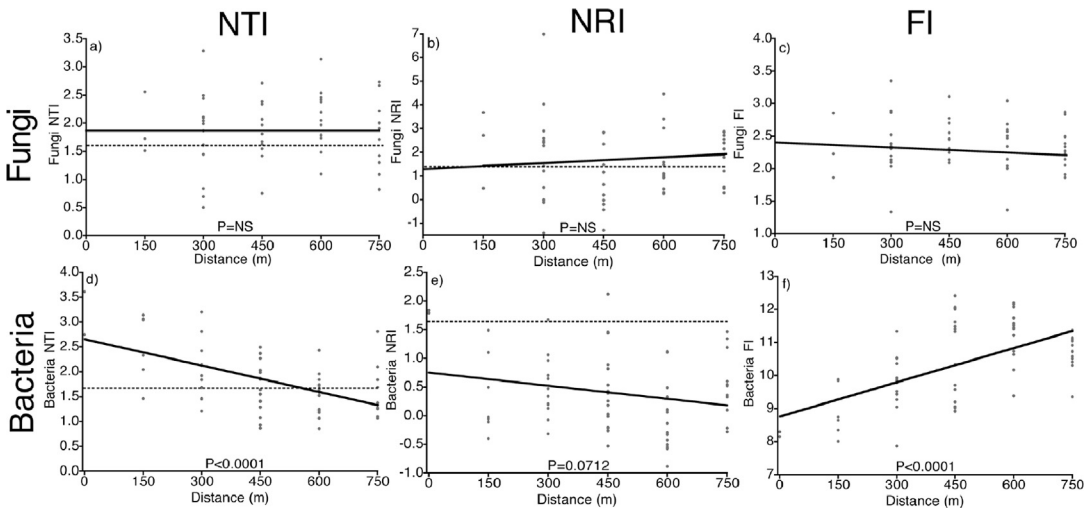

Fig. 1. Regression analyses of phylogenetic diversity of fungi and bacteria across the primary successional Lyman Glacier basin (distance from glacier terminus). The slopes of the lines of best fit are not different from zero with distance from Lyman glacier for fungi for (a) Nearest Taxon Index (NTI); (b) Net Relatedness Index (NRI); or (c) Faith's index of phylogenetic diversity (FI). These contrast with Bacteria that show a decrease in NTI over distance (d), marginal decrease in NRI (e) and increase in FI (f) over successional distance. Regression models are inserted within each panel and significant slope and intercept estimates (* = 0.05 ≥ P > 0.01; ** = 0.01 ≥ P > 0.001; *** = P ≤ 0.001). The dashed line for the NTI and NRI plots represents the significance threshold for phylogenetic clustering based on 1000 Monte Carlo simulations such that points above the dashed line are significantly clustered phylogenetically whereas those below are either not different from random or are phylogenetically over-dispersed.

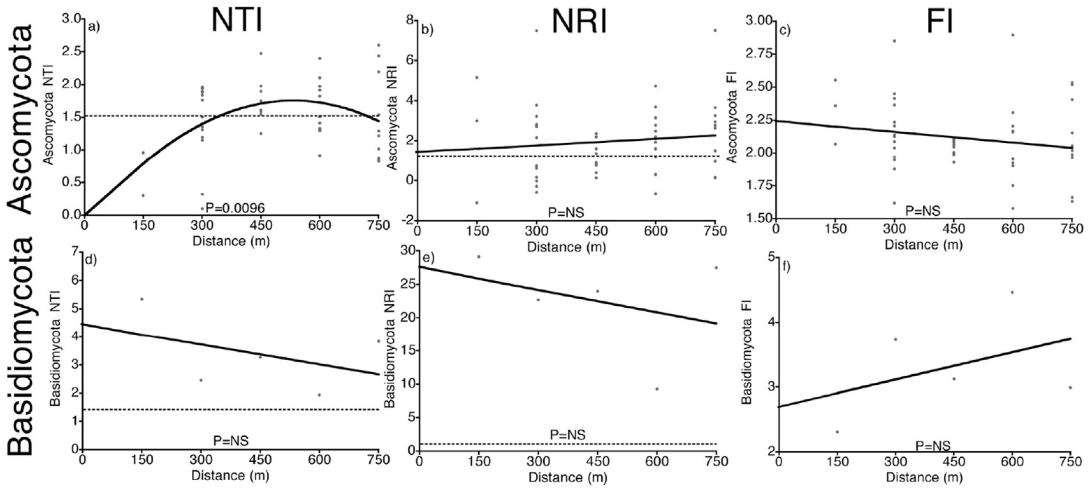

Fig. 2. Regression analyses of phylogenetic diversity measures of the fungal phyla Ascomycota (a – NTI; b – NRI; c – FI) and Basidiomycota (d – NTI; e – NRI; f – FI) show largely unchanging phylogenetic diversity over successional age. Regression models are inserted within each panel and significant slope and intercept estimates highlighted with asterisks (* = 0.05 ≥ P > 0.01; ** = 0.01 ≥ P > 0.001; *** = P ≤ 0.001). The dashed line for the NTI and NRI plots represents the significance threshold for phylogenetic clustering based on 1000 Monte Carlo simulations such that points above the dashed line are significantly clustered phylogenetically whereas those below are either not different from random or phylogenetically over-dispersed.

successional age. This contrasts with the strong fungal community turnover seen previously with successional age at this site (Brown and Jumpponen, 2014) suggesting that even though rampant species turnover occurs, species replacement is by members that are similarly distributed across the fungal phylogeny (perhaps even con-generics). In contrast, bacterial phylogenetic diversity responded strongly. Bacteria shift from phylogenetically clustered communities toward over-dispersed 'terminal' communities that include phylogenetically more distant taxa. This shift in phylogenetic structure suggests that early successional substrate has fewer niches that select for a phylogenetically more similar bacterial consortia. As the forefront ecosystem develops, more and unique niches open facilitating the co-occurrence of more dissimilar bacteria. Admittedly, this study and most studies on deglaciated primary succession involve detailed analyses of one glacier forefront and may thus be site-dependent. Ideally, multiple glacier forefronts would be queried that share similar allochthonous progagule and nutrient inputs but such cross-forefield studies are exceedingly rare and often logistically prohibitive.

Analyses of the whole fungal community indicate that approximately half the samples were phylogenetically clustered compared to randomly generated trees, a proportion unchanging over the chronosequence. Similarly, fungi within the phylum Basidiomycota did not markedly change in phylogenetic clustering over the chronosequence and were consistently phylogenetically clustered. This suggests that the basidiomycetes across the Lyman Glacier forefield possess highly similar niche attributes and that the soil ecosystem has low niche diversity for the Basidiomycota allowing only closely related taxa with similar environmental tolerances to establish and persist. Interestingly, only Ascomycota had

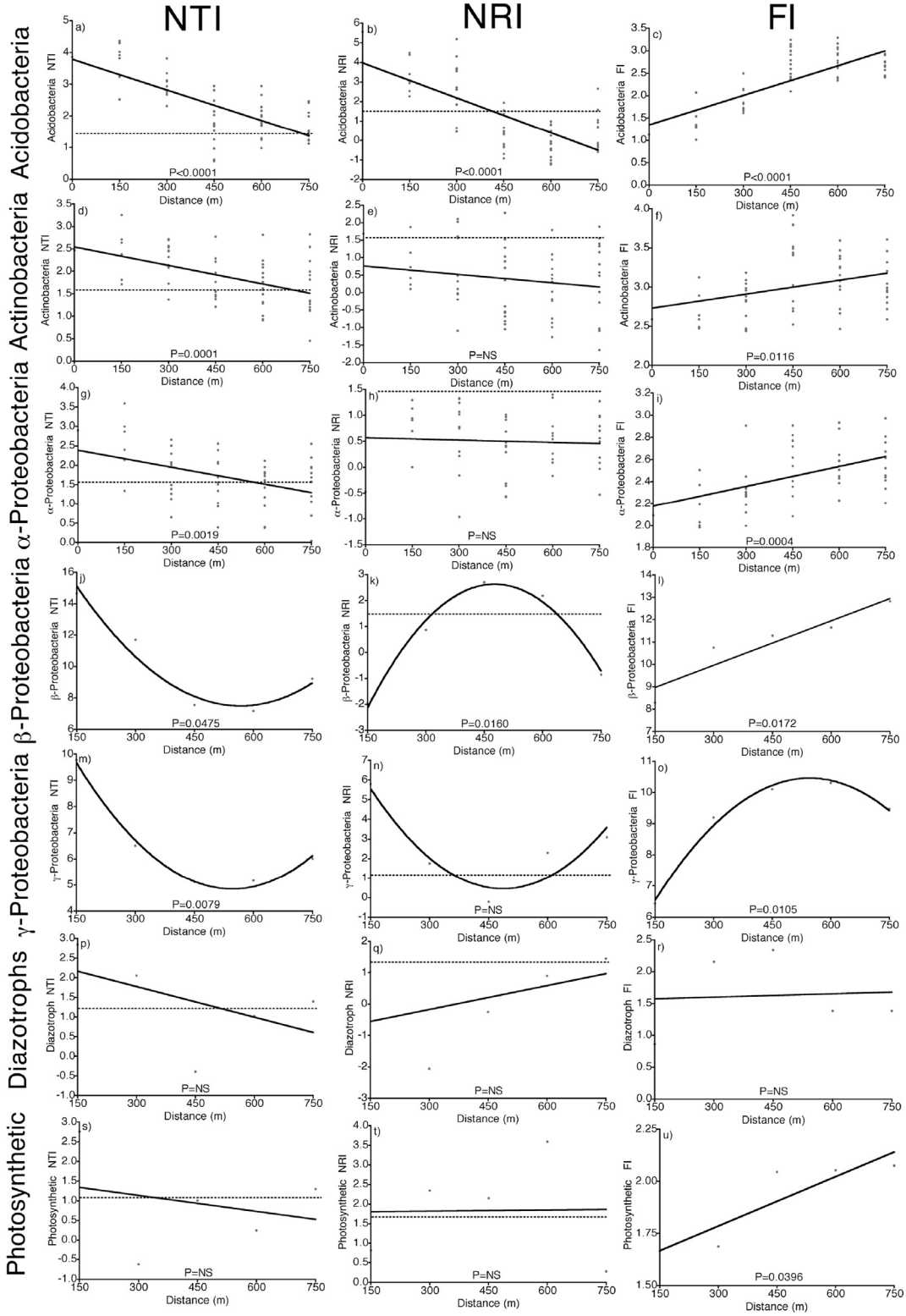

Fig. 3. Regression analyses of phylogenetic diversity measures of bacterial phyla, classes, or functional groups with distance from glacier terminus. Shown are Acidobacteria (a – NTI; b – NRI; c – FI), Actinobacteria (d – NTI; e – NRI; f – FI), α-Proteobacteria (g – NTI; h – NRI; i – FI), β-Proteobacteria (j – NTI; k – NRI; l – FI), γ-Proteobacteria (m – NTI; n – NRI; o – FI), Diazotrophic bacteria (p – NTI; q – NRI; r – FI) and photosynthetic bacteria (s – NTI; t – NRI; u – FI). Regression models are inserted within each panel and significant slope and intercept estimates ($^{*} = 0.05 \geq P > 0.01$; $^{**} = 0.01 \geq P > 0.001$; $^{***} = P \leq 0.001$). The dashed line for the NTI and NRI plots represents the significance threshold for phylogenetic clustering based on 1000 Monte Carlo simulations such that points above the dashed line are significantly clustered phylogenetically whereas those below are either not different from random or phylogenetically over-dispersed. Significance line (dashed line) for NTI for both β-Proteobacteria and γ-Proteobacteria are not depicted as all points fall well above threshold and are consistently clustered.

discernible patterns with successional age. The ascomycete Nearest Taxon Index (NTI) was best represented by curvilinear models (Fig. 2a) over the chronosequence. The early successional ascomycetes were randomly dispersed and their clustering increased with successional age until ~500 m from the glacier terminus after which point the clustering become less strong. The quadratic regression model implies that there is an initial phylogenetic convergence of the ascomycete community (NTI) followed by a later divergence. Reasons for this remain unclear but may be best explained by low-lying areas of the Lyman Glacier forefield at ~500 m from the glacier terminus that also possess distinct plant communities (Jumpponen et al., 2012). These depressions may alter both hydrology and microclimate at this site. These analyses highlight how different components of the fungal community follow different rules during early community assembly. Whilst basidiomycetes, and fungi in general, neither phylogenetically converge nor diverge, ascomycetes were phylogenetically structured during succession and initially converged (0 m–~500 m). This distinction between the Ascomycota and other fungi may in part be explained by the greater diversity and sequence abundance within the Ascomycota.

Analyses of the bacteria offer clear contrasts between bacteria and fungi. Whereas fungi had no discernible changes in phylogenetic diversity over successional time, unmistakable changes occur within Bacteria. The bacterial NTI and FI estimates responded strongly to distance from the glacier terminus; these trends were also marginally significant for NRI. In general, bacterial NTI and NRI decreased with a related increase in FI over time. The increasing FI is in accordance with our previous results showing a linear increase of bacterial richness with successional age (Brown and Jumpponen, 2014). Interestingly, clustering was more obvious for all bacteria and many of the less-inclusive phylogenetic groups at the terminal nodes (NTI) than for the deeper nodes (NRI). The stronger phylogenetic clustering at terminal nodes suggests that these bacterial communities are very closely related, perhaps at the genus level, further evidence for conserved niche space early on the chronosequence with niche divergence with increasing successional age.

Perhaps the most interesting observations emerge when the bacterial successional dynamics are queried at a lower phylogenetic resolution (phyla or classes). These dynamics seem as diverse as bacteria themselves. The strong response of the Acidobacteria that shadows the patterns found for all bacteria coupled with their great abundance in these soils suggest strongly that these bacteria are likely driving the overall community dynamics. Much about the Acidobacteria remains unknown, but these common soil-borne bacteria are generally thought to be oligotrophic (Fierer et al., 2007) and may be proficient in acquiring recalcitrant limiting nutrients from various substrata (Jangid et al., 2013), characteristics that are crucial for survival in primary successional systems where available nutrients are limiting. As a more diverse Acidobacterial community establishes, NTI and NRI predictably drop with a concomitant increase in FI. Interestingly, Actinobacteria and α-Proteobacteria also responded similarly to the whole bacterial community: while NTI declined and FI increased, NRI for these bacteria did not change. Unlike Acidobacteria that thrive in low nutrient conditions similar to those found in glacier forefronts, the copiotroph-oligotroph spectrum may not be applicable to the Actinobacteria and α-Proteobacteria (Fierer et al., 2007) because these taxa did not shift in abundances with carbon availability. We interpret this to mean that the Actinobacteria and α-Proteobacteria are strongly structured early in the chronosequence and respond to relaxing environmental constraints. Actinobacteria and α-Proteobacteria are ubiquitous; the negative regression of NTI with distance from the glacier can perhaps be best explained by arrival and establishment of closely related Actinobacterial or α-Proteobacterial taxa that diverge as a result of pedogenesis and ecosystem transformations that open new niches for more distantly related Actinobacteria or α-Proteobacteria. Contrastingly, both β-Proteobacteria and γ-Proteobacteria have a curvilinear relationship with distance from the glacier terminus with maxima or minima at around 600 m. This intriguing similarity with ascomycotetes may suggest that similar subtle shifts environmental conditions drive the community dynamics within these groups.

We also attempted to gain further insights into ecosystem processes by specifically targeting two fundamentally important and largely evolutionary conserved functional groups: the diazotrophs and photosynthetic bacteria. Primary successional environments suffer from extreme nutrient limitations, primarily nitrogen. Vitousek et al. (1987) demonstrated nitrogen-fixing plants preferentially invade young successional soils and this natural increase in nitrogen alters ecosystem developmental trajectories in volcanic soils. Experimental nutrient amendments demonstrated that nitrogen is the most limiting to plant establishment and concurrent ecosystem development (Vitousek et al., 1993). Similarly, Nemergut et al. (2007) demonstrated that bacteria establish ecosystem processes prior to any visible vegetation and that nutrient amendments to barren early successional soils shift bacterial community structure to resemble those found during later succession (Knelman et al., 2014). In our analyses, the diazotrophic and photosynthetic functional groups remained stable over time with the exception of FI for the photosynthetic bacteria. These results suggest that the selected functional traits are little structured during succession, despite their importance in early development of ecosystem processes (Schmidt et al., 2008). This is particularly interesting given the tight phylogenetic conservation of nitrogen fixation; this suggests that functional groups that are crucial for initial establishment of ecosystem processes are compositionally and phylogenetically stable during the early ecosystem development, especially early during the ecosystem development.

Our data strongly suggest that in order to better understand successional dynamics and ecosystem development, analyses of less inclusive levels of taxonomic hierarchy can provide far greater insight into ecosystem and community development than focusing on whole kingdom patterns. Comparing Kingdoms or Domains, such as Fungi and Eubacteria, as a whole does not allow decoupling of which community members drive any observed dynamics. While we are far from fully understanding the metabolic roles of individual species in complex microbial systems, analyses of phylogenetically conserved metabolic traits (N-fixation, photosynthetic activity, wood decomposition, or utilization of other recalcitrant substrates) may yield great insights into functional aspects of community assembly during early primary succession. The commonly observed increases/decreases in NTI (particularly within the Ascomycota, Acidiobacteria, Actinobacteria and α-Proteobacteria) and lack of strong responses in NRI suggest common community reordering with new but closely related community members. This may represent a type of fine-tuning or optimizing of communities for harsh and slightly shifting environmental conditions. We argue that greater insights can be gained from analyses that concurrently target lower and higher phylogenetic ranks, functional groups, or perhaps even target taxa with well-defined functional capabilities in order to provide more comprehensive understanding into succession and assembly dynamics.

Conflicts of interest

The authors declare no conflict of interest.

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