Twenty years of research on fungal–plant interactions on Lyman Glacier forefront – lessons learned and questions yet unanswered

Ari JUMPPONEN[a,*], Shawn P. BROWN[a], James M. TRAPPE[b], Efrén CÁZARES[b], Rauni STRÖMMER[c]

[a]Division of Biology, Kansas State University, Manhattan, KS 66506, USA
[b]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331, USA
[c]Department of Environmental Sciences, University of Helsinki, Lahti 15140, Finland

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ABSTRACT

Retreating glaciers and the periglacial areas that they vacate produce a harsh environment of extreme radiation, nutrient limitations and temperature oscillations. They provide a model system for studying mechanisms that drive the establishment and early assembly of communities. Here, we synthesize more than 20 yr of research at the Lyman Glacier forefront in the North Cascades Mountains, comparing the results and conclusions for plant and fungal communities. Compared to plant communities, the trajectories and processes of fungal community development are difficult to deduce. However, a combination of high throughput sequencing, more revealing experimental designs, and phylogenetic community analyses provide insights into mechanisms that shape early microbial communities. While the inoculum is likely to be randomly drawn from regional pools and accumulates over time, our data provide no support for increases in richness over time since deglaciation, as is commonly observed for plant communities. Re-analyses of existing datasets suggest that microbial, and particularly fungal, communities are insensitive to time since substrate exposure from underneath the retreating glacier, but are responsive to plant establishment both in biomass and community composition. Further research on functional aspects, organismal activity, or ecosystem services in early successional environments will provide deeper appreciation of the dynamics of these communities.

Introduction

Many alpine glaciers reached their glacial maximum during the Little Ice Age in the mid-19th century (Egli *et al.* 2001) and have been retreating over the past century and a half at increasing rates (Hodge *et al.* 1998; Dyurgerov & Meier 2000; Pelto 2006). Glacial retreat exposes a mineral substrate that is devoid of organic legacies and often deficient in mineral nitrogen (Matthews 1992; Tscherko *et al.* 2003; Strauss *et al.* 2009), factors that globally limit ecosystem productivity (Vitousek *et al.* 1997). These nutrient limitations are often combined with extreme fluctuations of daily temperatures, limited barriers against wind damage, poor water retention and high irradiation, all of which may limit plant establishment and survival (Stocklin & Baumler 1996; Jumpponen *et al.* 1999b; Lichter 2000; Jones & del Moral 2009). Additionally,

glaciers and their forefronts most often occur at high latitudes and altitudes, characterized by short growing seasons and substantial winter snow cover. Together, these abiotic stressors make primary successional forefronts challenging environments to research, but they also provide unique opportunities to study the early assembly of communities (Cázares et al. 2005).

Many mechanisms controlling primary succession in plant communities have been recently clarified (Walker & del Moral 2003; Pickett et al. 2009) and older theories reevaluated (Clements 1916; Connell & Slatyer 1977). Some of these insights have shifted views on community assembly processes in terrestrial ecosystems. For example, early successional community development seems not to depend on deterministic colonization by pioneering species but rather combines stochastic dispersal and establishment controls (Fastie 1995; del Moral 2009) that are later amended by deterministic processes such as biotic, competitive and facilitative controls (del Moral 2009).

Long-term studies in glacier forefronts are rare because of their remote locations and short accessible seasons, dictated by high altitudes and latitudes. Instead, glacier forefronts have often been subjected to a chronosequence approach (space-for-time substitution), in which distance from the glacier terminus is considered as a proxy for time since exposure (Pickett 1989; Cázares et al. 2005; Walker et al. 2010). While suffering from potential correlations between position in the chronosequence and substrate chemistry, fluctuations in weather or climatic conditions, distance to propagule sources, or other environmental parameters (Fastie 1995; Walker et al. 2010), the forefronts benefit from providing a single location wherein substrates of different ages can be observed in a relatively homogeneous environment (Matthews 1992; Jumpponen et al. 1998; Cázares et al. 2005; Raffl et al. 2006).

In this contribution, we reflect on and synthesize more than 20 yr of research at a glacier forefront in Washington State's North Cascades Mountains, which contain more than 700 glaciers (Post et al. 1971). Similarly to glaciers globally (Hodge et al. 1998; Dyurgerov & Meier 2000), glaciers in the North Cascades have been receding in recent decades (Pelto 2006, 2011). To optimize our choice of a forefront environment, we explored several glaciers to find one that would best serve as the long-term study site. Our criteria included (1) reasonable accessibility, (2) a forefront with relatively little elevational change, (3) a subalpine habitat to allow establishment of ectomycorrhizal (EcM), ericoid mycorrhizal (ErM) and arbuscular mycorrhizal (AM) hosts and fungi as well as typically nonmycorrhizal plants, (4) a north-south orientation to minimize diurnal shade effects along the length of the forefront, and (5) accessibility for potential animal vectors of mycorrhizal spores. These criteria also served well for microbial and molecular studies. One forefront was almost ideal in all respects: the Lyman Glacier, in the Glacier Peak Wilderness Area, Wenatchee National Forest (Freeman 1941). The glacier and its recession have been photographed off and on since the late 19th Century (Freeman 1941) and later by periodic aerial photography by the U.S. Forest Service, including new colour aerial photographs taken specifically for our use. These resources allowed a reasonable description of the glacier's recession and chronosequence (Jumpponen et al. 1998).

Many of our studies test hypotheses about the establishment and succession of communities. We have focused on the role of mycorrhizal fungi in primary plant succession and subsequent secondary succession. Early studies by Reeves et al. (1979) showed that in a desert ecosystem with a severely disturbed, secondary successional habitat, the early plant invaders were nonmycorrhizal species, whereas plants that dominated the adjacent nondisturbed system mostly formed associations with AM fungi. Reeves et al. (1979) also reviewed literature on primary succession of volcanic islands that showed early plant communities to be nonmycorrhizal. They hypothesized that the early nonmycorrhizal invaders were poor competitors and were therefore rapidly replaced when mycorrhizal hosts could establish after AM inoculum had entered the disturbed site. These AM hosts were argued to be better competitors and produced additional AM inoculum in the soil to enable other mycorrhizal plants to establish. Allen et al. (2005) reached similar conclusions on primary successional tephra resulting from the Mount St. Helens eruption. One of our early driving questions was whether or not a glacier forefront would exhibit similar primary and secondary successions.

While glacier systems differ in numerous characteristics and the conclusions may be context dependent, many general trends and patterns have proved consistent (Tscherko et al. 2003; Orwin et al. 2006). Here, we use our research on the Lyman Glacier forefront, which spans more than 20 yr, as a model. We first briefly review patterns of plant community establishment and trajectories, then compare plant and fungal communities. Finally, we identify critical areas that have received little attention and propose approaches to address them in fungal and/or microbial systems.

Community assembly and ecological filtering

As a general framework, we rely on assembly rules used in community ecology (Cole 1983; Hunt 1991). This community assembly model integrates traits and life histories and their contribution to organismal environmental tolerances (Jumpponen & Egerton-Warburton 2005). Factors determining successful establishment are considered as abiotic and biotic filters that select community components from local, regional and ecologically suited species pools (Weiher & Keddy 1995, 2001; Booth & Swanton 2002). Local and regional propagule pools determine candidate species with potential for being included in the community, but the ecological filters (Weiher & Keddy 1995; Weiher & Keddy 2001) remove candidates that fail to establish or persist under the present local environment (Grubb 1977; Southwood 1988). Combined, the assembly rules outline constraints on the selection of communities from larger potential constituent species pools (Weiher & Keddy 2001). These assembly rules can also elucidate processes that produce communities present in a habitat or environment (Drake et al. 1993; Booth & Swanton 2002). In conclusion, assembly rules and ecological filtering are particularly useful in successional ecology, because they account for both stochastic (e.g., distribution of suitable establishment sites and random distribution of propagules in seed and spore

banks) and deterministic factors (*e.g.*, facilitative and competitive processes once communities establish) in a unified framework.

Plant community dynamics

Plant establishment

Microsites (safe sites, *sensu* Harper *et al.* 1961), where plants establish in primary succession, are not random (Jumpponen *et al.* 1999b), but are characterized by assemblages with low levels of organization (Robbins & Matthews 2009) where the importance of biotic and abiotic controls shifts over time (del Moral 2009). As a result of environmental heterogeneity, some microsites trap larger propagule numbers or may be more favourable for germination and seedling establishment (Harper *et al.* 1965; Oswald & Neuenschwander 1993; Titus & del Moral 1998; Jumpponen *et al.* 1999b).

Studies in glacier forefronts have identified surface depressions, nearby rocks and coarse surface particles as the characteristics that are positively associated with plant occurrence (Stocklin & Baumler 1996; Jumpponen *et al.* 1999b; Schlag & Erschbamer 2000; Erschbamer *et al.* 2001; Jones & del Moral 2005a). The underlying mechanisms include shade, increased soil moisture, and changes in surface temperatures (Jumpponen *et al.* 1999b; Schlag & Erschbamer 2000; Jones & del Moral 2005a), suggesting the importance of physical environmental amelioration. Plant establishment is also controlled by seed (propagule) availability and size (Primack & Miao 1992; Turnbull *et al.* 2000; Clark *et al.* 2007) determined by the surrounding communities, relative fecundities of component species and the distance of safe sites from the propagule sources (Schlag & Erschbamer 2000; Jones & del Moral 2009). Safe sites may facilitate seed trapping, thereby increasing their resident seed banks (Jumpponen *et al.* 1999b; Jones & del Moral 2009).

Safe site requirements for seed germination and seedling establishment appear to be similar among plant species during early primary succession (del Moral & Wood 1993; Jumpponen *et al.* 1999b; Jones & del Moral 2005a; Walker *et al.* 2006). Shifts in safe site preferences are likely to indicate relaxation of abiotic environmental stressors, leading to dominant competitive interactions and greater requirements for differentiation in resource use in later succession. Established plants simultaneously compete for resources with newly establishing seedlings. Consequently, the balance between competition and facilitation may be difficult to determine (Chapin *et al.* 1994; Jumpponen *et al.* 1998). Nitrogen fixing plants, such as infrequent *Alnus* and *Lupinus* spp. on the Lyman Glacier forefront, have been argued to be particularly important because they reduce nitrogen limitation, in addition to improving water retention in the developing soils and providing shade to reduce irradiation (Walker *et al.* 2003).

Patterns of plant community development

To describe plant community diversity and heterogeneity at Lyman Glacier, we recorded plant species (see Supplementary Table S1 for vascular plant checklist) in clusters of four 0.25 m² sub-plots located at 20 m intervals along four equidistant transects for a total of 228 1 m² sampling units. From these data we calculated plant species richness (S), Shannon−Wiener diversity (H′), and evenness (−H′/lnS) and compared community compositions using Nonmetric Multidimensional Scaling (NMS, McCune & Grace 2002). To construct a metric for community heterogeneity, we estimated similarity of community compositions among plots using percent similarity (PS) as described by del Moral & Jones (2002), and regressed PS against distance from the glacier terminus to test whether or not our communities tended to converge or diverge over time (del Moral & Jones 2002; del Moral 2009).

In contrast to Coleman Glacier on Mount Baker, also in Washington state (Jones & del Moral 2005b), the most recently deglaciated plots at Lyman Glacier were devoid of vegetation, and nonvegetated plots occurred even near the terminal moraine (Fig 1). The first individuals of the nonmycorrhizal *Luzula piperi* and *Saxifraga ferruginea*, and the ectomycorrhizal *Abies lasiocarpa*, had established after approximately 15 yr since deglaciation, suggesting a limited control of establishment by the mycorrhizal habit (compare with Read 1991) and limited dependence on the presence of mycorrhizal fungal propagules (see also Reeves *et al.* 1979; Collier & Bidartondo 2009). Overall, the species richness at the 1 m² scale was low, but was comparable with that observed at Coleman Glacier (Jones & del Moral 2005b). At Lyman Glacier, richness reached a maximum of 6.25 ± 0.82 (mean ± SD) near the terminal moraine. The highest number of species recorded in any one plot was 10.

As is common in early primary succession (Reiners *et al.* 1971; Matthews 1992), plant species richness and diversity increased with time since deglaciation, as evidenced by our linear regression analyses (Fig 2A, B). Evenness was low (0.23 ± 0.08) and constant with time since deglaciation. These patterns stem from sparse plant occurrence near the glacier terminus and increasing, but still heterogeneous, cover near the terminal moraine. Although increasing plant richness and biomass are common observations in chronosequence studies (Reiners *et al.* 1971; Matthews 1992), our observations contrast with those reported at a similar scale on Coleman Glacier (Jones & del Moral 2005b): dense canopies have not established at Lyman Glacier forefront in the first hundred years since glacial retreat.

In order to classify plant community types (CTs), the 228 experimental units were assigned into seven clusters by complete linkage clustering (see del Moral & Jones 2002) using JMP (version 7.01, SAS Institute, Cary, North Carolina), so that the clusters retained ~75 % of the information at the plot level (Supplementary Fig S1). The seven CTs were characterized by two species that were most strongly associated with that cluster (Fig 1, inset). These seven CTs represent largely overlapping communities (Supplementary Fig S2a and S2b) indicating gradual shifts in community composition. Evaluation of the CT frequencies along the Lyman Glacier forefront permits an assessment of community trajectories and their dynamics. Nonvegetated patches and early establishing nonmycorrhizal plants (CTs 1 and 4, with *Juncus drummondii* and *Saxifraga ferrugina*) occur commonly but in variable frequencies throughout the forefront. The common communities near the terminal moraine are CTs 2 and 7, comprised primarily of plant species frequent in the montane parkland habitat outside the forefront, including the AM-forming
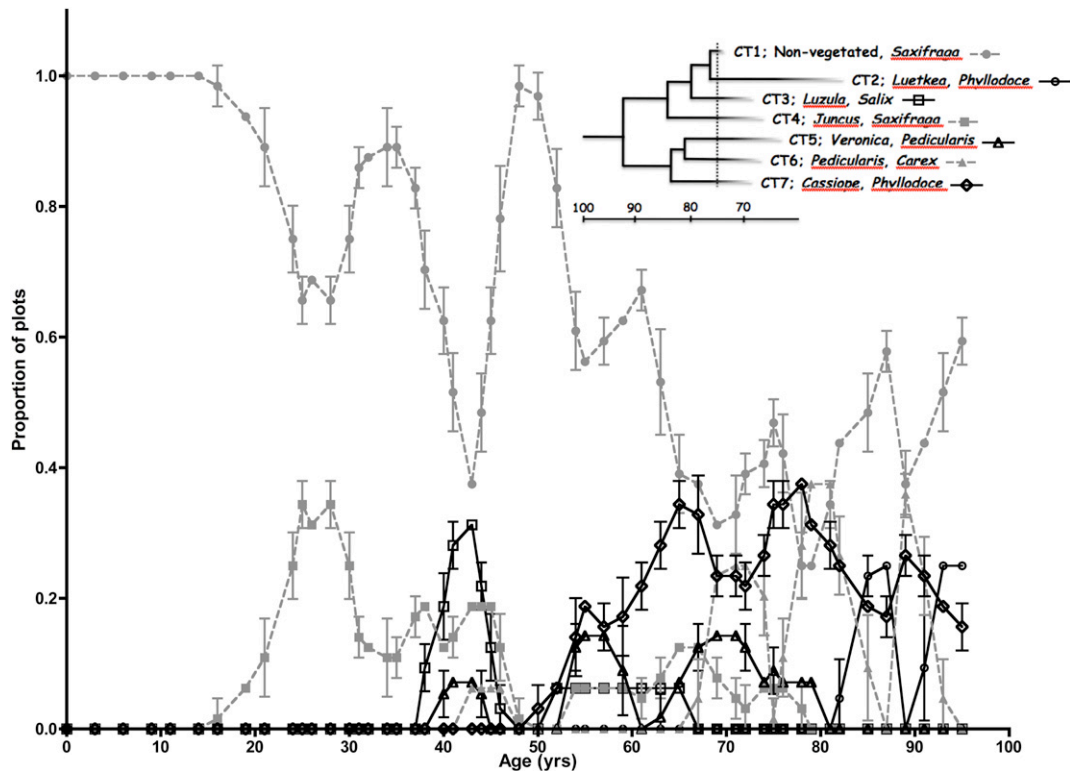
Fig 1 – Plant community type (CT) frequency dynamics as a function of time since substrate exposure in the forefront of the receding Lyman Glacier. The inset identifies the dominant components of the CTs based on two-way complete linkage clustering shown in Supplementary Fig S1. Note that the CT1 – characterized by nonmycorrizal *Saxifraga* and nonvegetated, open areas – is frequent through the chronosequence even close to the terminal moraine.

Luetkea pectinata and the ErM plants *Cassiope mertensiana* and *Phyllodoce empetriformis*. None of the EcM Pinaceae (*A. lasio-carpa*, *Larix lyalii* and *Tsuga mertensiana*), common in forested patches among the alpine meadows adjacent to the forefront, are frequent enough to weigh in the community assignments.

These analyses suggest that not only the successional trajectories, but also the environmental heterogeneity, in this system dictate the CT occurrence. To exemplify, CTs 3, 5 and 6, comprising *L. piperi* and *Salix phylicifolia*, *Veronica wormsk-joldii* and *Pedicularis groenlandica*, and *P. groenlandica* and *Carex scopularis*, respectively, occur mainly in depressions and near creeks that likely maintain high soil moisture. This empha-sizes the difficulties of applying the space-for-time chro-nosequence approach (Walker *et al.* 2010), but simultaneously indicates the sensitivity of CT analyses to primary determi-nants of plant community development.

Deterministic vs. stochastic processes in the plant community succession

Plant community convergence towards a terminal (climax) state community was integral in early concepts of succession (Clements 1916). If the deterministic processes were to increase with succession, then plant community composition should follow a predictable trajectory (del Moral 2009) and become more homogeneous over successional time (Leps & Rejmanek 1991). However, convergence towards a stable community (Pickett 1989) may depend on factors such as

heterogeneity of the local species or their propagule pools, strength of the biological legacies, importance of priority effects, and sensitivity to conditions during initial stages of succession (Walker *et al.* 2010). We discuss below trajectories based on ordination analyses and changes in community similarities among plots along the chronosequence.

In our plant community ordination analyses, NMS scores for the first axis increased linearly, suggesting a predictable trajectory along this axis (Fig 3) as suggested in early concepts of succession (Clements 1916). Changes in the other two axes were not linear but were best characterized by regression models that included quadratic terms (data not shown). When standard deviations of NMS axis scores were analyzed by linear regression, they increased linearly for axis 1 (Fig 4) and axis 2 (not shown). While communities may be changing to include a greater component from surrounding late succes-sional communities, the increasing standard deviations suggest a strong contribution of stochastic processes or priority effects in almost a century since deglaciation (del Moral 2009). More importantly, the increasing standard deviations suggest that these periglacial plant communities are characterized by community divergence rather than convergence during early succession.

To further illuminate the determinism in the plant communities, we calculated percent similarity (PS) for each group of plots representing equal time since deglaciation (see del Moral & Jones 2002). In contrast to increasing
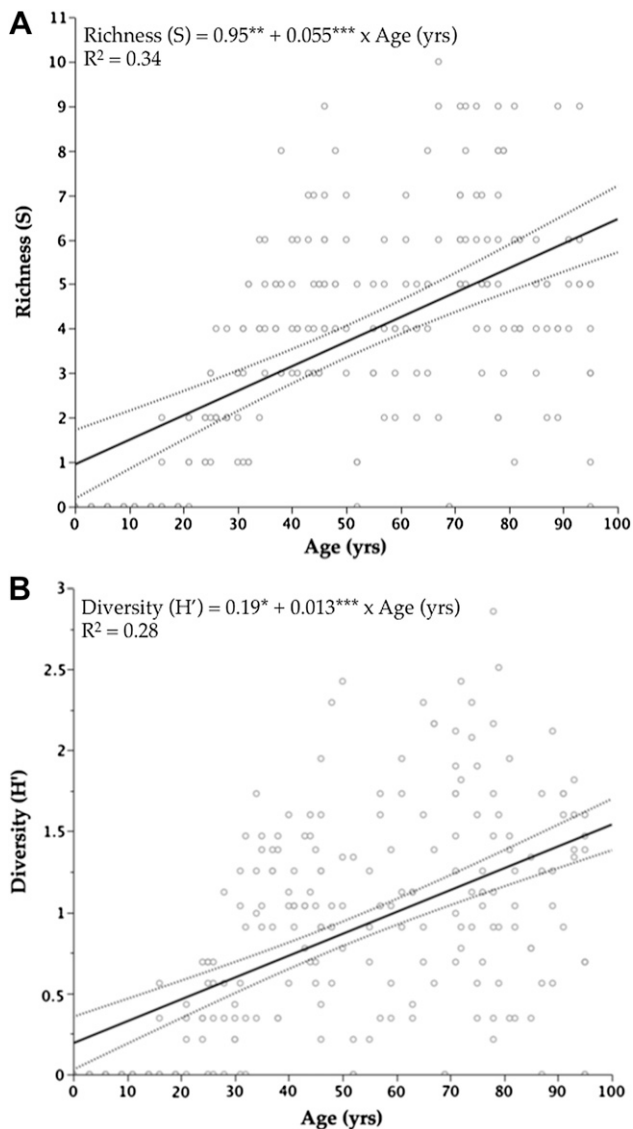
A

Richness (S) = 0.95** + 0.055*** x Age (yrs)
$R^2 = 0.34$

Richness (S) — Age (yrs)

B

Diversity (H') = 0.19* + 0.013*** x Age (yrs)
$R^2 = 0.28$

Diversity (H') — Age (yrs)

Fig 2 — Plant community dynamics as a function of time since substrate exposure in the forefront of the receding Lyman Glacier: (A) plant species richness (S) increases, the inset identifies the linear regression model with significant intercept and slope terms; (B) Shannon–Wiener diversity (H') increases, the inset identifies the linear regression model with significant intercept and slope terms. $^{ns}P > 0.05$; $^*P \leq 0.05$; $^{**}0.01 \leq P < 0.05$; $^{***}\leq 0.001$.

Mean Axis1 = -1.20*** + 0.021*** x Age (yrs)
$R^2 = 0.66$

Mean Axis1 — Age (yrs)

Fig 3 — Mean Nonmetric Multidimensional Scaling (NMS) Axis 1 scores of the plant community types (CTs) along the Lyman Glacier forefront increase with time since deglaciation. The inset identifies the linear regression model with significant intercept and slope terms. The linear increase in the NMS scores suggests a successional trajectory in this primary successional system and is likely correlated with increasing abundance of the CTs with component species from the adjacent montane meadow communities. Notation as in Fig 2.

St Dev Axis1 = 0.02^{ns} + 0.01*** x Age (yrs)
$R^2 = 0.41$

St Dev Axis1 — Age (yrs)

Fig 4 — Mean Nonmetric Multidimensional Scaling (NMS) Axis 1 score standard deviations of the plant community types (CTs) along the Lyman Glacier forefront increase with time since deglaciation. The inset identifies the linear regression model with a significant slope term. The increase in the NMS score standard deviations suggests a divergence of the plant communities in this primary successional system and is likely a result of stochastic plant establishment processes and heterogeneous distribution of the CTs along the successional chronosequence. Notation as in Fig 2.

determinism in the primary successional system at Mount St. Helens in Washington state (del Moral 2009), our PS estimates decreased with time since deglaciation (Fig 5). This observation corroborates the analyses of the NMS standard deviations and points to stochastic processes. These findings are primarily attributable to the occurrence of a large number of different CTs and the persistence of the early successional communities near the terminal moraine. In sum, we conclude that plant communities at Lyman Glacier site are diverging rather than converging in these stages of succession.
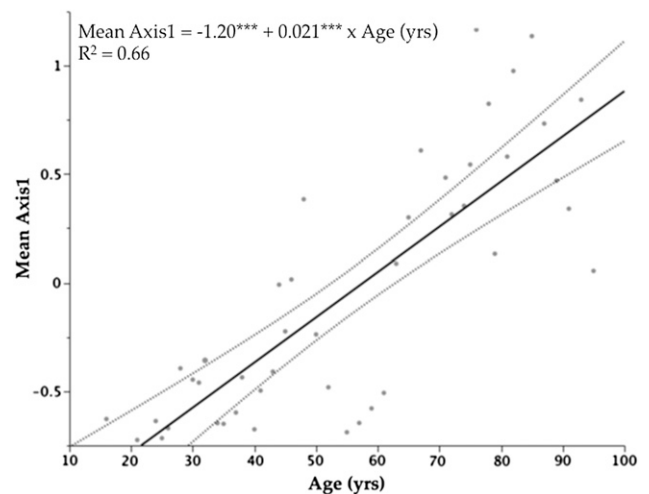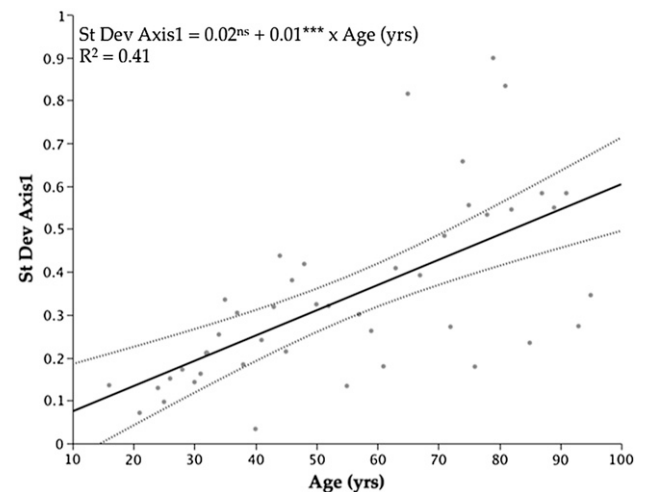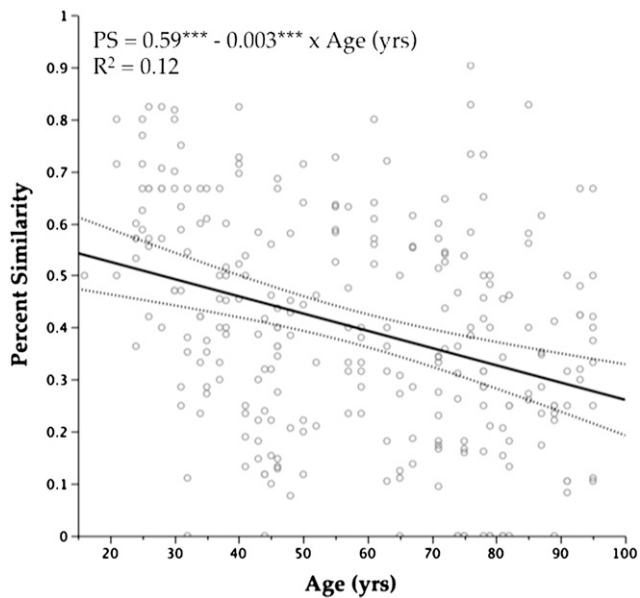
PS = 0.59*** - 0.003*** x Age (yrs)
R² = 0.12

Fig 5 − **Percent similarity (PS) of the plant communities along the Lyman Glacier forefront decline with time since deglaciation. The inset identifies the linear regression model with significant intercept and slope terms. The decrease in the PS corroborates divergence of the plant communities in this primary successional system, as shown in Fig 4. Notation as in Fig 2.**

Fungal community dynamics

Sources and distribution of fungal propagules
Establishment of fungal propagule banks from atmospheric sources in newly exposed substrates is little debated, and the distribution of these banks has received little attention. It is uncertain whether microtopological characteristics similar to those for plant safe sites accumulate microbes as a result of reduced air velocities or water flow. The Baas–Becking hypothesis (Martiny *et al.* 2006) states that because of their small size and enormous numbers, microbial propagules are everywhere and that the environment selects from the established propagule banks. In contrast to such environmentally based niche models, recent work suggests that microbial communities, like those of macroscopic eukaryotes, may suffer from dispersal limitations. They may thus be assembled by stochastic immigration, establishment, and local extinction events (Peay *et al.* 2010).

The Lyman Glacier forefront is surrounded by bountiful sources of seeds and spores plus living fragments of plants and fungi. Its east and west sides are bounded by cliffs and benches leading to ridges with meadows and subalpine trees. The lowermost moraine has adjacent meadows and groups of conifers and willows. Only at the head of the glacier are there cliffs and crags with little vegetation. The plant and fungal propagules may be dispersed onto the forefront by abiotic factors such as wind, rain, flowing water and landslides, as well as by biotic means, such as by birds, mammals and arthropods. The continuous input of material and propagules

onto the glacier and periglacial areas is evidenced by frequent deposits of woody debris covered with lichens.

A propagule rain falls on the glacier and its forefront during wind and rain storms, particularly when epigeous, *i.e.* aboveground fruiting bodies, release spores. Consequently, propagules of EcM fungi are likely to be available to hosts in early succession. This was implicit in the EcM conifer seedlings appearing early in the primary succession. Collier & Bidartondo (2009) reported on heathlands where EcM spores are rare, and where pines and birches establish and persist for at least a year after germination without EcM formation, in effect waiting for EcM inoculum to arrive. This is not likely to be the case on the Lyman Glacier forefront: all Pinaceae sampled, even first year seedlings, were colonized by EcM fungi (Cázares *et al.* 2005). The two systems differ fundamentally: the heathlands studied by Collier & Bidartondo (2009) have high soil organic matter that provides water retention and nutrients. In contrast, the periglacial outwash from Lyman Glacier has little organic matter to sustain seedlings while they wait for EcM colonization to establish. These phenomena are inferred from relatively few seedling samples, because the depletion of seedlings by intensive sampling would have drastically interfered with early successional stages.

Early EcM colonization seems plausible, because soil recently exposed from under the ice contained DNA of *Laccaria*, a genus that commonly fruits among the EcM trees on the forefront (Jumpponen 2003). Older soil, *i.e.* at the terminal moraine, contained a greater diversity of fungi than the recently exposed soils. Propagule numbers also increase over time, as shown by a study using EcM-forming *Pinus contorta* as bait (Trowbridge & Jumpponen 2004). These results corroborate the presence of fungal propagules in recently deglaciated substrates, although their numbers were low. Accordingly, it is likely that − in addition to falling on the exposed glacial till − spores also land on the glacier, to be washed into crevasses and ultimately into the water and soil emerging from under the ice. This propagule dispersal likely establishes a resident propagule bank soon after deglaciation. Microtopology, which determines air and water flow, is likely to distribute the propagule pools unevenly across the landscape, thereby establishing safe sites that differ in their spore numbers and community compositions.

Although relatively infrequent on the glacier forefront, EcM willows and conifers and the accumulated leaf litter under their crowns support fruiting EcM fungi. Repeated searches over more than a decade on the forefront revealed that in all, sporocarps of only 13 species of EcM fungi were present, with only a few of the nearly 70 species present in the adjacent subalpine parklands appearing on the forefront (Jumpponen *et al.* 1999a). Conversely, several species recorded on the forefront were not found in the surrounding areas (Jumpponen *et al.* 2002). The most abundant genera were *Cortinarius*, *Inocybe* and *Laccaria*. These genera were also common in primary successional, upper montane and subalpine habitats under *Salix* on volcanic substrates on Mt. Fuji, Japan (Nara *et al.* 2003a). Two species, *Cortinarius decipiens* and *Inocybe lacera*, were observed to be locally abundant at both Lyman Glacier and Mt. Fuji. A major propagule source of *I. lacera* and *Cortinarius* and *Laccaria* spp. at Lyman Glacier was

the old outwash meadow/willow habitat, which is probably several thousand years old, immediately below the terminal moraine (J. Trappe, unpublished data).

Nara *et al.* (2003b) determined that in early succession, the fungi forming EcM were generally the same as those that fruited aboveground, and that the diversity of fungal species gradually increased with plant community development. Based on fruit body data, this was also true to a degree at Lyman Glacier (Jumpponen *et al.* 2002): no EcM fruit bodies were observed at up to 300 m from the terminus, two species fruited at 300−400 m, and eight at 800−900 m. Nara *et al.* (2003b) found, from molecular analysis, that several EcM fungi were resupinate; the same is largely true of the Lyman Glacier forefront (Trowbridge & Jumpponen 2004). No fruit bodies of resupinate fungi have been recorded at Lyman Glacier (Jumpponen *et al.* 2002), but spores of hypogeous fungi reliant on animal mycophagy for dispersal were detected on the forefront in the scats of yellow-pine chipmunks, hoary marmots, pikas and mule deer (Cázares & Trappe 1994). None of the fungi represented by those spores were found fruiting on the forefront. Indeed, only one fruiting of a hypogeous fungus was recorded over the several years of sampling: a tiny new species, *Hymenogaster glacialis*, fruited among willow mycorrhizas (Cázares & Trappe 1990). Because of its small size (<3−6 mm width), it could have been more common but overlooked. Nonetheless, the dispersal of hypogeous fungal spores is stochastic, depending on animal vectors that establish spore deposits locally enriched for particular species. Animals can also disperse spores of epigeous EcM fungi. For example, deer are effective in the inoculation of pines with both hypogeous and epigeous fungi in primary successional habitats in coastal sand dunes (Ashkannejhad & Horton 2006).

AM fungi increase steadily after disturbance in many early successional ecosystems, indicating successional dynamics of these communities (Allen & Allen 1980; Gemma & Koske 1990; Koske & Gemma 1997; Greipsson & El-Mayas 2000). However, compared to old-field or dune systems, patterns of AM establishment in a montane glacial system may be stochastic, dictated by the movement of soil containing inoculum (Warner *et al.* 1987). We inferred stochastic AM spore deposition from mycorrhizal colonization, because spores of Glomeromycota were rarely encountered (Cázares *et al.* 2005). On the Lyman Glacier forefront, dispersal is likely to be mainly on the feet or in the scats of visiting animals (see also Allen *et al.* 1984; Warner *et al.* 1987), but small avalanches or landslides from cliffs and benches that border the forefront, or perhaps water flow from established plant communities on lateral moraines, might also be responsible for propagule movement. These dispersal mechanisms are likely because AM plants were often more common at the edges than in the centre of the forefront. The most recently exposed substrates (15−25 yr) supported no or few AM plants, but otherwise no clear patterns were evident, further evidencing the stochastic nature of AM spore dispersal. Helm *et al.* (1996) reported that AM colonization and spores were infrequent and showed no particular patterns on the forefront of an Alaskan low-elevation glacier, further evidencing spore dispersal limitation in these habitats.

Our analyses above show that CTs with *Cassiope* and *Phyllodoce* increase late on the Lyman Glacier chronosequence. While arrival of the ErM fungi could not be observed directly, ErM colonization of the susceptible hosts gradually increased over time since soil exposure (Cázares *et al.* 2005), suggesting gradual buildup of an inoculum pool, probably from air- and/or ungulate-borne propagules. Similarly to ErM, dark septate endophyte (DSE) colonization gradually increased over the chronosequence regardless of the mycorrhizal habit of the host (Cázares *et al.* 2005). These observations parallel those of Peay *et al.* (2010): dispersal seems a key control of fungal community composition in early successional stages.

Patterns of fungal community development
To attempt fungal community analyses comparable with those on plants, we reanalyzed an EcM dataset (Trowbridge & Jumpponen 2004) and two others on soil communities (Jumpponen 2003, 2007). These data may not be directly comparable with those for plants because of differences in data volumes and recording. However, they illustrate contrasts and similarities between fungal and plant communities and identify further research needs.

In contrast to plant communities, where we directly estimated development as a function of time since deglaciation, we decoupled the effects of plant establishment and time since deglaciation on fungal communities. Trowbridge & Jumpponen (2004) described EcM communities of *Salix* spp. and observed that richness, diversity and evenness estimates were rather insensitive to distance from the glacier terminus as well as to the willow canopy microenvironment. Whereas the community metrics were unresponsive, individual components (EcM morphotypes identified through ITS-RFLP and sequencing) showed preferences for canopy or inter-canopy environments, while some increased in frequency with time since deglaciation. Trowbridge & Jumpponen (2004) argued that these results suggested niche preferences related to organic legacies associated with soil development.

To compare successional trajectories between fungal and plant communities, we used complete linkage clustering of the EcM morphotype data. We analyzed these data by either including nonmycorrhizal root tips in the community type (CT) constituents or excluding them from the constituents. With the nonmycorrhizal tips included, two clusters were identified that retained 71.2 % of the plot-level variability (not shown). The clusters were defined by two of the three morphotypes assigned to the Sordariales in the original analyses (Trowbridge & Jumpponen 2004). Exclusion of the non-mycorrhizal root tips revealed five clusters that retained 74.3 % of total variability at the plot level (Supplementary Fig S3). These community analyses were uninformative with respect to time since deglaciation or canopy environment because three of the five clusters occurred only once, twice, and three times in the 30 samples. The most common cluster, occurring 19 times, was defined by its heterogeneity rather than by its unifying community components: the common morphotypes (two Sordariales and two Cortinariaceae; Supplementary Fig S3) were common constituents of this CT.

These analyses highlight fundamental issues that complicate fungal community analyses. A ten-fold increase in sampling, similar to that for plant communities, would be

difficult to achieve by microscopic EcM morphotyping and molecular identification. Moreover, while the EcM communities may not be as diverse as those in soil (Buée et al. 2009), their complete characterization, even in an early successional system with limited diversity, would be difficult. However, the development of high throughput parallel sequencing tools (Cardenas & Tiedje 2008) combined with DNA-tagging (Meyer et al. 2008) might solve the issues necessary to differentiate among the root-inhabiting fungal communities.

Deterministic vs. stochastic processes in fungal community succession

We analyzed combined datasets (from Jumpponen 2003, 2007) to determine if we could draw inferences on the responses of fungal communities and/or their components to time since deglaciation. In common with Emerson & Gillespie (2008), our analyses assume immigration and environmental filtering, not speciation, to be the fundamental processes of community assembly in early succession. The scale of a glacier forefront is unlikely to lead to cladogenic speciation, mainly because the large allochthonous source populations maintain geneflow into the periglacial environment.

To test whether the EcM communities associated with Salix were undergoing community-level convergence or divergence, we analyzed the NMS axis score standard deviations and estimated PS as described above for plant communities. When the standard deviations of the NMS axis scores were analyzed by linear regression, with vs. without the terms that would account for the canopy environment plus interaction terms, the axis score standard deviations showed no significant effects of either time since deglaciation or canopy ($P > 0.15$ for all effect tests using ANOVA or t-tests for null hypotheses that parameter estimates equal zero). Similarly, none of the simple linear or multiple regression models analyzed to test the effects of Salix canopies and time since deglaciation on PS differed significantly for convergence or divergence ($P > 0.30$ for all effect tests using ANOVA or t-tests for null hypotheses that parameter estimates equal zero). In other words, we found no support for patterns similar to those observed for plant communities.

We also targeted general fungal communities associated with soils underneath Salix canopies vs. intercanopy areas using an approach similar to that described above. In these analyses, we tested whether differential phylogenetic clustering between the two canopy environments could be detected or whether phylogenetic patterns could be visualized in relation to time since deglaciation. Using the data matrices comparing within-sample phylogenetic distances among the experimental units, we sought stochastic vs. deterministic trajectories of fungal communities. To do this, we re-analyzed Small Sub-Unit (SSU) sequences of the ribosomal RNA gene from two clone library studies (Jumpponen 2003, 2007) characterizing soils collected from canopy or intercanopy locations along the chronosequence. The soil samples originated from 0 to 900 m from the glacier terminus and included 15 from underneath Salix canopies (Jumpponen 2007) and 17 intercanopy samples from across the chronosequence (Jumpponen 2003). We reanalyzed representative sequences from the two studies and MUSCLE-aligned them using Geneious Pro 5.3.4 (Biomatters Ltd., Chirstchurch, New Zealand).

The alignments were analyzed by the neighbor joining (NJ) method and the tree distance matrix obtained was analyzed with Fast Unifrac (Hamady et al. 2010) with a Principal Coordinates Analysis (PCoA). The PCoA scores obtained for the first three axes (representing 20.6 %, 11.0 %, and 7.1 % of the variability) and the NJ distance matrix were used to analyze community similarity described above by use of both the standard deviations of PCoA scores and within-sample mean NJ distances to test hypotheses on community convergence or divergence.

Analyses of PCoA axis scores indicated that Axis 1 and Axis 3 scores did not vary significantly with time since deglaciation or canopy condition. In contrast, Axis 2 seemed to drive the observed patterns in a full model (goodness of fit; $F_{3,28} = 6.4361$, $P = 0.0019$), with significant effects of time since deglaciation and canopy condition. In this model, Axis 2 PCoA scores increased with time since deglaciation (Fig 6; $F_{3,28} = 4.829$, $P = 0.0364$), indicating a trajectory in fungal community composition based on SSU sequence data. We interpret these data to indicate that the communities are shifting somewhat predictably and incorporating different community members to lead to such a trajectory. In contrast, we found no evidence for canopy effects on community composition. Analyses of standard deviations of PCoA axis scores similar to those that we used for plant communities indicated that Axis 3 score standard deviations decreased with distance from the glacier terminus (Fig 7; full model goodness of fit $F_{3,28} = 9.1490$, $P = 0.0002$), suggesting phylogenetic convergence with time since deglaciation. However, standard deviations for Axis 1 and 2 scores showed no response to time since deglaciation, suggesting minor convergent patterns.

These analyses of the PCoA scores and their standard deviations provide a starting point for asking which
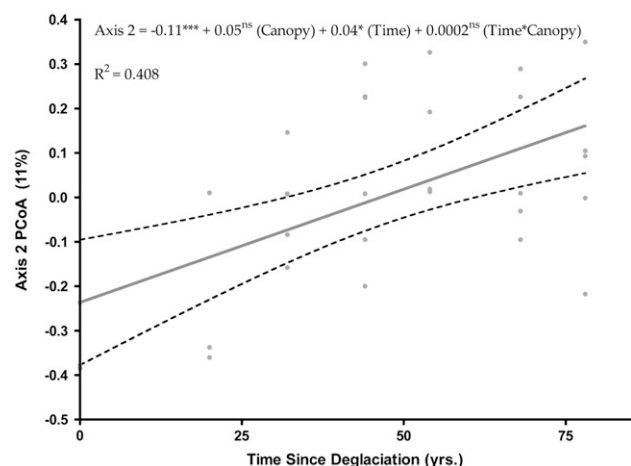

Axis 2 = $-0.11^{***} + 0.05^{ns}$ (Canopy) + 0.04^{*} (Time) + 0.0002^{ns} (Time*Canopy)

$R^2 = 0.408$

Fig 6 – **Principal Coordinates Analysis (PCoA) Axis 2 scores for fungal communities along the Lyman Glacier forefront increase with time since deglaciation. The inset identifies the linear regression model with significant terms for intercept and slope but not for canopy position or interaction. The linear increase in the PCoA scores suggests a successional trajectory in this primary successional system. Notation as in Fig 2.**
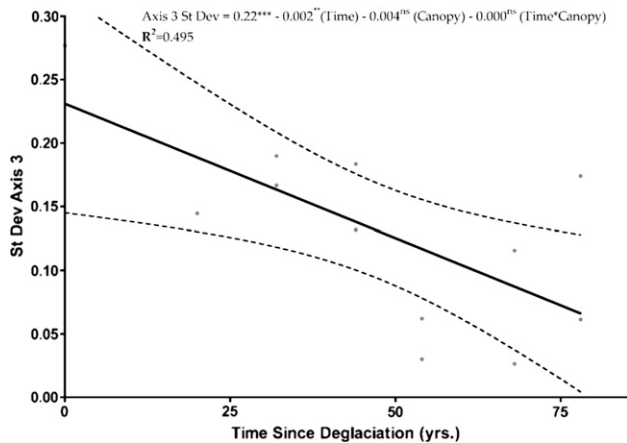
Fig 7 – Principal Coordinates Analysis (PCoA) Axis 3 score standard deviations for fungal communities along the Lyman Glacier forefront decrease with time since deglaciation. The inset identifies the linear regression model with significant terms for intercept and slope but not for canopy position or interaction. The linear decrease in the standard deviations suggests a community convergence over time in this primary successional system. Notation as in Fig 2.
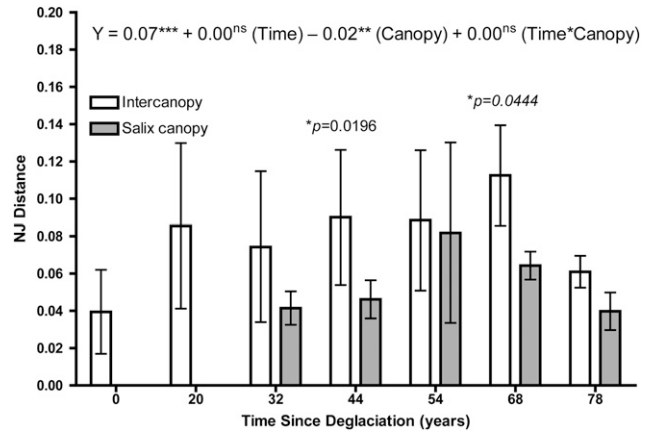

Fig 8 – Neighbor Joining (NJ) distances (mean ± SD) at the sample level for fungal communities along the Lyman Glacier forefront are greater in the canopy samples than in the intercanopy samples. The inset identifies the linear regression model with significant terms for intercept and canopy position but not for slope or the interaction. The difference between the canopy and intercanopy samples suggests a canopy soil community convergence relative to intercanopy soils in this primary successional system. The two groups of samples with different NJ distances are indicated by *P*-values (oneway ANOVA) above the bars. Notation as in Fig 2.

organisms may be enriched in the late primary successional soils. Our earlier studies indicate that EcM fruit bodies (Jumpponen *et al.* 2002), root-associated propagules (Trowbridge & Jumpponen 2004), and the root colonization of various hosts (Cázares *et al.* 2005) increase over successional time. However, only *Tomentella* (Thelephoraceae; GenBank Accession DQ092920) increased in frequency with time since deglaciation and none decreased. Others also increased with time since deglaciation, including taxa with various non-symbiotic life history strategies such as *Mycoacia* (Merulinaceae; DQ873636) and *Pulvinula* (Pyronemataceae; U62012). Thus, the increasing trajectory of Axis 2 PCoA scores and convergence indicated by declining Axis 3 PCoA score standard deviations corroborate results of our earlier studies and evidence a relationship of plant establishment with subsequent fungal community enrichment.

In addition to PCoA, we analyzed the SSU RNA gene NJ distance matrix to directly compare sample-level distances in *Salix* canopy and intercanopy soils along the forefront chronosequence. These analyses tested if these communities show a random assembly from the regional propagule pools and would therefore possess equal ability to establish across the periglacial substrates. Alternatively, if environmental filters shape the establishing communities, the communities should show phylogenetic structuring – perhaps related to the establishment of few organisms that share similar preferences for habitat or hosts but are not competitively exclusive. In contrast to the analyses of Axis 2 PCoA scores, fungal community NJ distances did not vary with time since deglaciation ($F_{1,28} = 0.0068$, $P = 0.93$), indicating that the fungal communities are not strongly affected by time of substrate exposure from underneath the glacier (Fig 8). However, judging from these analyses, fungal communities of canopy

soils had significantly lower NJ distances than those from non-vegetated areas (Fig 8; $F_{2,28} = 11.1$, $P = 0.0022$). The nonsignificant interaction term indicates canopy effects regardless of position in the periglacial chronosequence. We conclude that fungal communities converge in the canopy soils relative to nonvegetated soils and that plant establishment homogenizes soil fungal communities. Although the results of our PCoA and NJ analyses are incongruent, a simple explanation for them is that, while the intercanopy communities may be a random draw of propagules, the canopy soils in the sparsely-vegetated early plant communities are enriched for fungi that rely on host photosynthates or litter. To better tie this to the community assembly model that we introduced previously (Jumpponen & Egerton-Warburton 2005), this convergence can be argued to result from the selection of fungi from the local propagule pool based on their compatibility with *Salix* and its canopy soil environment. This is best exemplified by foliage-associated *Coniochaeta* (GenBank Accession GQ154624), the only taxon positively associated with canopies: it did not respond to time since deglaciation in our analyses.

The convergence inferred from the NJ distances corroborates our earlier PLFA studies (Ohtonen *et al.* 1999), in which microbial communities differed between canopy and intercanopy soils. These results emphasize the importance of plant establishment in shaping early microbial communities (Ohtonen *et al.* 1999). While compositional changes with time since deglaciation were not obvious, the samples collected underneath established plants were more tightly clustered in the NMS ordination than those collected from nonvegetated

areas (see Fig 2 in Ohtonen et al. 1999). Although those data do not permit a more thorough convergence analysis, we propose that, in combination with our NJ distance analyses, plants tend to homogenize microbial communities and select subsets of compatible micro-organisms from stochastic propagule pools.

These conclusions parallel results from replicated soil transfers on the Lyman forefront (Jumpponen et al. 1998). In those studies, small mesh bags were filled with willow canopy soil and an equal number with intercanopy soils. Seeds of P. contorta, which occurs on the forefront, were planted in each bag. Separate holes to accommodate one bag of each soil type were dug under canopies and in intercanopy sites. Seedling emergence and survival were recorded 8 weeks after sowing and were low in all treatments (two soil types × two locations), but were on average twice as high in the canopy soil transferred to intercanopy spaces than in other treatments. Canopies thus appeared to suppress emergence/survival and intercanopy soils were similarly adverse in that respect. The superior performance of seedlings in canopy soil in intercanopy sites indicates that the combined enhancement of nutrients and microbes provided greater benefit when the suppressive effects of the canopies themselves were removed.

A comparison of the successional trajectories of plant and microbial communities

Our synthesis of the work conducted on the Lyman Glacier forefront over more than two decades has identified gaps in our current understanding of the fundamental processes of early microbial community assembly. Early propagule accumulation and the processes that control it prior to community assembly are poorly understood for both microbial and plant communities. A framework of safe microsites for plant seed accumulation and seedling establishment exists, but the presence of such sites for microbial communities remains uncertain. Mechanisms that control selection of active microbial community members from established propagule banks are equally unclear. The difficulty of selecting an appropriate scale for the 'microbial landscape' and its ecology complicates these issues.

Our parallel analyses of plant and microbial communities highlight some clear and distinct dissimilarities between the two, because plant establishment modifies soil chemistry and the physical environment. To account for plant controls of microbial communities, our analyses aimed to decouple the effects of substrate exposure and plant establishment. Early plant community development at Lyman Glacier forefront can be characterized by increasing species richness and community divergence over time since deglaciation, but analyses of the microbial communities provide no strong evidence for similar changes. In contrast, establishing plants appear to homogenize soil-inhabiting microbial communities in our periglacial system, and these effects may strengthen over time. Our analyses of microbial communities using PLFA and SSU sequence data, which broadly characterize microbial communities, show a tighter clustering of the samples obtained from soils underneath plant canopies compared to soils from open, nonvegetated areas. In sum, the fungal and microbial communities of canopy soils converge relative to intercanopy soils.

We hope that these analyses and discussions will arouse interest in further analyses of microbial community trajectories, as well as in patterns and mechanisms of microbial community divergence and convergence. Microbial communities tend to be orders of magnitude more complex than those of plants and animals, but the extreme environments in primary successional ecosystems limit microbial richness. Furthermore, recent studies provide insights into microbial ecosystem functions and processes, particularly those preceding the establishment of plants (Schmidt et al. 2008), as well as communities that may be comprised of unexpected and/or novel constituents (Nemergut et al. 2007; Freeman et al. 2009). Our analyses here, and those published elsewhere, evidence that successional processes of microbial communities are unlikely to be successfully modelled after those of plants, although the influence of plants on microbial communities must be accounted for after plant establishment. We conclude that microbial communities and their dynamics express novel community level processes with important consequences for plant community development: this field is wide open for research, especially with newly available molecular and statistical techniques.

Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.funeco.2012.01.002.

REFERENCES

Allen EB, Allen MF, 1980. Natural re-establishment of vesicular-arbuscular mycorrhizae following stripmine reclamation in Wyoming. *Journal of Applied Ecology* **17**: 139–147.

Allen MF, Macmahon JA, Andersen DC, 1984. Reestablishment of Endogonaceae on Mount St. Helens - survival of residuals. *Mycologia* **76**: 1031–1038.

Allen MF, Crisafulli CM, Morris SJ, Egerton-Warburton LM, MacMahon JA, Trappe JM, 2005. Mycorrhizae and Mount St. Helens: story of a symbiosis. In: Dale VH, Swanson FJ, Crisafulli CM (eds), *Ecological Responses to the 1980 Eruption of Mount St. Helens*. Springer Verlag, New York, pp. 221–231.

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.

Booth BD, Swanton CJ, 2002. Assembly theory applied to weed communities. *Weed Science* **50**: 2–13.

Buée M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F, 2009. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist* **184**: 449–456.

Cardenas E, Tiedje JM, 2008. New tools for discovering and characterizing microbial diversity. *Current Opinion in Biotechnology* **19**: 544–549.

Cázares E, Trappe JM, 1990. Alpine and sub-alpine fungi of the Cascade Mountains. 1. *Hymenogaster glacialis* sp. nov. *Mycotaxon* **38**: 245–249.

Cázares E, Trappe JM, 1994. Spore dispersal of ectomycorrhizal fungi on a glacier forefront by mammal mycophagy. *Mycologia* **86**: 507–510.

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.

Clark CJ, Poulsen JR, Levey DJ, Osenberg CW, 2007. Are plant populations seed limited? A critique and meta-analysis of seed addition experiments. *American Naturalist* **170**: 128–142.

Clements FE, 1916. *Plant Succession: An Analysis of the Development of Vegetation*. Carnegie Institution of Washington, Washington, D.C.

Cole BJ, 1983. Assembly of mangrove ant communities - patterns of geographical distribution. *Journal of Animal Ecology* **52**: 339–347.

Collier FA, Bidartondo MI, 2009. Waiting for fungi: the ectomycorrhizal invasion of lowland heathlands. *Journal of Ecology* **97**: 950–963.

Connell JH, Slatyer RO, 1977. Mechanisms of succession in natural communities and their role in community stability and organization. *American Naturalist* **111**: 1119–1144.

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, Wood DM, 1993. Early primary succession on a barren volcanic plain at Mount St. Helens, Washington. *American Journal of Botany* **80**: 981–991.

del Moral R, Jones C, 2002. Vegetation development on pumice at Mount St. Helens, USA. *Plant Ecology* **162**: 9–22.

Drake JA, Flum TE, Witteman GJ, Voskuil T, Hoylman AM, Creson C, Kenny DA, Huxel GR, Larue CS, Duncan JR, 1993. The construction and assembly of an ecological landscape. *Journal of Animal Ecology* **62**: 117–130.

Dyurgerov MB, Meier MF, 2000. Twentieth century climate change: evidence from small glaciers. *Proceedings of the National Academy of Sciences of the United States of America* **97**: 1406–1411.

Egli M, Mirabella A, Fitze P, 2001. Clay mineral formation in soils of two different chronosequences in the Swiss Alps. *Geoderma* **104**: 145–175.

Emerson BC, Gillespie RG, 2008. Phylogenetic analysis of community assembly and structure over space and time. *Trends in Ecology & Evolution* **23**: 619–630.

Erschbamer B, Kneringer E, Schlag RN, 2001. Seed rain, soil seed bank, seedling recruitment, and survival of seedlings on a glacier foreland in the Central Alps. *Flora* **196**: 304–312.

Fastie CL, 1995. Causes and ecosystem consequences of multiple pathways of primary succession at Glacier Bay, Alaska. *Ecology* **76**: 1899–1916.

Freeman O, 1941. The recession of Lyman Glacier, Washington. *The Journal of Geology* **49**: 764–771.

Freeman KR, Martin AP, Karki D, Lynch RC, Mitter MS, Meyer AF, Longcore JE, Simmons DR, Schmidt SK, 2009. 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.

Gemma JN, Koske RE, 1990. Mycorrhizae in recent volcanic substrates in Hawaii. *American Journal of Botany* **77**: 1193–1200.

Greipsson S, El-Mayas H, 2000. Arbuscular mycorrhizae of *Leymus arenarius* on coastal sands and reclamation sites in Iceland and response to inoculation. *Restoration Ecology* **8**: 144–150.

Grubb PJ, 1977. Maintenance of species richness in plant communities - importance of regeneration niche. *Biological Reviews of the Cambridge Philosophical Society* **52**: 107–145.

Hamady M, Lozupone C, Knight R, 2010. Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. *ISME Journal* **4**: 17–27.

Harper JL, McNaughton IH, Sagar GR, Clatworthy JN, 1961. Evolution and ecology of closely related species living in the same area. *Evolution* **15**: 209–227.

Harper JL, Williams JT, Sagar GR, 1965. The behavior of seeds in soil. 1. The heterogeneity of soil surfaces and its role in determining the establishment of plants from seed. *Journal of Ecology* **53**: 273–286.

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

Hodge SM, Trabant DC, Krimmel RM, Heinrichs TA, March RS, Josberger EG, 1998. Climate variations and changes in mass of three glaciers in western North America. *Journal of Climate* **11**: 2161–2179.

Hunt GL, 1991. Occurrence of polar seabirds at sea in relation to prey concentrations and oceanographic factors. *Polar Research* **10**: 553–559.

Jones CC, del Moral R, 2005a. Effects of microsite conditions on seedling establishment on the foreland of Coleman Glacier, Washington. *Journal of Vegetation Science* **16**: 293–300.

Jones CC, del Moral R, 2005b. Patterns of primary succession on the foreland of Coleman Glacier, Washington, USA. *Plant Ecology* **180**: 105–116.

Jones CC, del Moral R, 2009. Dispersal and establishment both limit colonization during primary succession on a glacier foreland. *Plant Ecology* **204**: 217–230.

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, 2007. Soil fungal communities underneath willow canopies on a primary successional glacier forefront: rDNA sequence results can be affected by primer selection and chimeric data. *Microbial Ecology* **53**: 233–246.

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, Cazares E, 1999a. Ectomycorrhizal fungi in Lyman Lake Basin: a comparison between primary and secondary successional sites. *Mycologia* **91**: 575–582.

Jumpponen A, Väre H, Mattson KG, Ohtonen R, Trappe JM, 1999b. Characterization of 'safe sites' for pioneers in primary succession on recently deglaciated terrain. *Journal of Ecology* **87**: 98–105.

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

Koske RE, Gemma JN, 1997. Mycorrhizae and succession in plantings of beachgrass in sand dunes. *American Journal of Botany* **84**: 118–130.

Leps J, Rejmanek M, 1991. Convergence or divergence - what should we expect from vegetation succession. *Oikos* **62**: 261–264.

Lichter J, 2000. Colonization constraints during primary succession on coastal Lake Michigan sand dunes. *Journal of Ecology* **88**: 825–839.

Martiny J, Bohannan B, Brown J, Colwell R, Fuhrman J, Green J, 2006. Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiology* **4**: 102–112.

Matthews JA, 1992. *The Ecology of Recently-Deglaciated Terrain: A Geoecological Approach to Glacier Forelands and Primary Succession*. Cambridge University Press, Cambridge.

McCune B, Grace JB, 2002. *PC-ORD Multivariate Analysis of Ecological Data*. MjM Software Design, Glendale Beach, Oregon.

Meyer M, Stenzel U, Hofreiter M, 2008. Parallel tagged sequencing on the 454 platform. *Nature Protocols* **3**: 267–278.

Nara K, Nakaya H, Hogetsu T, 2003a. Ectomycorrhizal sporocarp succession and production during early primary succession on Mount Fuji. *New Phytologist* **158**: 193–206.

Nara K, Nakaya H, Wu BY, Zhou ZH, Hogetsu T, 2003b. Underground primary succession of ectomycorrhizal fungi in a volcanic desert on Mount Fuji. *New Phytologist* **159**: 743–756.

Nemergut DR, Anderson SP, Cleveland CC, Martin AP, Miller AE, Seimon A, Schmidt SK, 2007. Microbial community succession in an unvegetated, recently deglaciated soil. *Microbial Ecology* **53**: 110–122.

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.

Orwin KH, Wardle DA, Greenfield LG, 2006. Context-dependent changes in the resistance and resilience of soil microbes to an experimental disturbance for three primary plant chronosequences. *Oikos* **112**: 196–208.

Oswald BP, Neuenschwander LF, 1993. Microsite variability and safe site description for western larch germination and establishment. *Bulletin of the Torrey Botanical Club* **120**: 148–156.

Peay KG, Garbelotto M, Bruns TD, 2010. Evidence of dispersal limitation in soil microorganisms: isolation reduces species richness on mycorrhizal tree islands. *Ecology* **91**: 3631–3640.

Pelto MS, 2006. The current disequilibrium of North Cascade glaciers. *Hydrological Processes* **20**: 769–779.

Pelto MS, 2011. Methods for assessing and forecasting the survival of North Cascade, Washington glaciers. *Quaternary International* **235**: 70–76.

Pickett STA, 1989. Space for time substitutions as an alternative to long-term studies. In: Likens GE (ed), *Long-Term Studies in Ecology*. Springer, New York, pp. 110–135.

Pickett STA, Cadenasso ML, Meiners SJ, 2009. Ever since Clements: from succession to vegetation dynamics and understanding to intervention. *Applied Vegetation Science* **12**: 9–21.

Post A, Richardson DW, Rossellot FF, 1971. *Inventory of Glaciers in the North Cascades, Washington*. U.S. Department of the Interior Geological Survey Professional Paper 705-A, Washington D.C.

Primack RB, Miao SL, 1992. Dispersal can limit local plant distribution. *Conservation Biology* **6**: 513–519.

Raffl C, Mallaun M, Mayer R, Erschbamer B, 2006. Vegetation succession pattern and diversity changes in a glacier valley, Central Alps, Austria. *Arctic Antarctic and Alpine Research* **38**: 421–428.

Read DJ, 1991. Mycorrhizas in ecosystems. *Experientia* **47**: 376–391.

Reeves FB, Wagner D, Moorman T, Kiel J, 1979. Role of endomycorrhizae in revegetation practices in the semi-arid west. 1. Comparison of incidence of mycorrhizae in severely disturbed vs natural environments. *American Journal of Botany* **66**: 6–13.

Reiners WA, Worley IA, Lawrence DB, 1971. Plant diversity in a chronosequence at Glacier Bay, Alaska. *Ecology* **52**: 55–69.

Robbins JA, Matthews JA, 2009. Pioneer vegetation on glacier forelands in southern Norway: emerging communities? *Journal of Vegetation Science* **20**: 889–902.

Schlag RN, Erschbamer B, 2000. Germination and establishment of seedlings on a glacier foreland in the central Alps, Austria. *Arctic Antarctic and Alpine Research* **32**: 270–277.

Schmidt SK, Reed SC, Nemergut DR, Grandy AS, Cleveland CC, Weintraub MN, Hill AW, Costello EK, Meyer AF, Neff JC, Martin AM, 2008. The earliest stages of ecosystem succession in high-elevation (5000 metres above sea level), recently deglaciated soils. *Proceedings of the Royal Society B-Biological Sciences* **275**: 2793–2802.

Southwood TRE, 1988. Tactics, strategies and templets. *Oikos* **52**: 3–18.

Stocklin J, Baumler E, 1996. Seed rain, seedling establishment and clonal growth strategies on a glacier foreland. *Journal of Vegetation Science* **7**: 45–56.

Strauss SL, Ruhland CT, Day TA, 2009. Trends in soil characteristics along a recently deglaciated foreland on Anvers Island, Antarctic Peninsula. *Polar Biology* **32**: 1779–1788.

Titus JH, del Moral R, 1998. Seedling establishment in different microsites on Mount St. Helens, Washington, USA. *Plant Ecology* **134**: 13–26.

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

Tscherko D, Rustemeier J, Richter A, Wanek W, Kandeler E, 2003. Functional diversity of the soil microflora in primary succession across two glacier forelands in the Central Alps. *European Journal of Soil Science* **54**: 685–696.

Turnbull LA, Crawley MJ, Rees M, 2000. Are plant populations seed-limited? A review of seed sowing experiments. *Oikos* **88**: 225–238.

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman GD, 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* **7**: 737–750.

Walker LR, Clarkson BD, Silvester WB, Clarkson BR, 2003. Colonization dynamics and facilitative impacts of a nitrogen-fixing shrub in primary succession. *Journal of Vegetation Science* **14**: 277–290.

Walker LR, del Moral R, 2003. *Primary Succession and Ecosystem Rehabilitation*. Cambridge University Press, Cambridge.

Walker LR, Bellingham PJ, Peltzer DA, 2006. Plant characteristics are poor predictors of microsite colonization during the first two years of primary succession. *Journal of Vegetation Science* **17**: 397–406.

Walker LR, Wardle DA, Bardgett RD, Clarkson BD, 2010. The use of chronosequences in studies of ecological succession and soil development. *Journal of Ecology* **98**: 725–736.

Warner NJ, Allen MF, Macmahon JA, 1987. Dispersal agents of vesicular arbuscular mycorrhizal fungi in a disturbed arid ecosystem. *Mycologia* **79**: 721–730.

Weiher E, Keddy PA, 1995. Assembly rules, null models, and trait dispersion – new questions front old patterns. *Oikos* **74**: 159–164.

Weiher E, Keddy PA, 2001. *The Assembly Rules: Perspectives, Advances, Retreats*. Cambridge University Press, Cambridge, Massachusetts.