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

Ari Jumpponen\textsuperscript{a}, Shawn P. Brown\textsuperscript{a}, James M. Trappe\textsuperscript{b}, Efrén Cázares\textsuperscript{b}, Rauni Strömmer\textsuperscript{c}

\textsuperscript{a} Division of Biology, Kansas State University, Manhattan, KS66506, USA

\textsuperscript{b} Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR97331, USA

\textsuperscript{c} Department of Environmental Sciences, University of Helsinki, Lahti, 15140, Finland

* Corresponding author. Division of Biology, Kansas State University, Manhattan, KS66506, USA. Tel.: +1 785 532 6751; fax: +1 785 532 6653

Keywords: community assembly, community convergence, community divergence, community trajectory, establishment, glacier forefront, mycorrhizae, propagule
Retreating glaciers and the periglacial areas they vacate for organismal colonization produce a harsh environment of extreme radiation, nutrient limitations, and temperature oscillations. They provide a model system for studying mechanisms that drive establishment and early assembly of communities. Here, we synthesize more than twenty years of research at the Lyman Glacier forefront in the North Cascades Mountains, comparing the results and conclusions for plant and microbial communities. Compared to plant communities, the trajectories and processes of microbial community development are difficult to deduce. However, the combination of high throughput sequencing, more revealing experimental designs, and analyses of phylogenetic community provide insights into mechanisms that shape early microbial communities. While the inoculum is likely 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, particularly fungal, communities are insensitive to time since substrate exposure from underneath the retreating glacier but 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 for 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 (Dyurgerov & Meier 2000, Hodge et al. 1998, Pelto 2006). Glacial retreat exposes a mineral substrate void of organic legacies and often deficient in mineral nitrogen (Matthews 1992, Strauss et al. 2009, Tscherko et al. 2003), 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 (Jones & del Moral 2009, Jumpponen et al. 1999b, Lichter 2000, Stocklin & Baumler 1996). Additionally, glaciers and their forefronts most often occur at high latitudes and altitudes characterized by short growing seasons and substantial snow cover during the winters. Together, these abiotic stressors make the primary successional forefronts challenging environments but also provide unique opportunities to study early assembly of communities (Cázares et al. 2005).

Many mechanisms controlling primary succession in plant communities have been recently clarified (Pickett et al. 2009, Walker & del Moral 2003) and older theories (Clements 1916, Connell & Slatyer 1977) reevaluated. 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 (del Moral 2009, Fastie 1995) that later are 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 (Cázares et al. 2005, Pickett 1989, 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 homogenous environment (Cázares et al. 2005, Jumpponen et al. 1998, Matthews 1992, Raffl et al. 2006).

In this contribution, we reflect on and synthesize more than twenty years 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 (Dyurgerov & Meier 2000, Hodge et al. 1998), glaciers in the North Cascades have been receding in recent decades (Pelto 2006, 2011). To optimize our choice for 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 approached near ideal in all criteria: Lyman Glacier, in the Glacier Peak Wilderness Area, Wenatchee National Forest (Freeman 1941). The glacier and its recession had 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 color aerial photographs taken specifically for our use. These resources allowed a reasonable description of the glacier’s recession and chronosequence approaches to explore successional phenomena (Jumpponen et al. 1998).
Many of our studies test hypotheses on establishment and succession of communities. We have focused on the role of mycorrhizal fungi in primary plant succession and the 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 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 present similar primary and secondary succession.

While glacier systems differ in numerous characteristics and the conclusions may be context dependent, many general trends and patterns have proved consistent (Orwin et al. 2006, Tscherko et al. 2003). Here, we use Lyman Glacier forefront and our research spanning more than twenty years as a model. We first briefly review patterns of plant community establishment and trajectories, then compare plant and microbial communities. Much of our earlier work focused on mycorrhizal fungus communities; more recent studies broadly emphasize bacterial and fungal communities. Finally, from these cross-domain comparisons, 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 (Booth & Swanton 2002, Weiher & Keddy 1995, Weiher & Keddy 2001). 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 environmental (Grubb 1977, Southwood 1988). Combined, the assembly rules outline constraints on 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 (Booth & Swanton 2002, Drake et al. 1993). 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 characterized as 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

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

Safe site requirements for seed germination and seedling establishment appear similar among plant species during early primary succession (del Moral & Wood 1993, Jones & del Moral 2005a, Jumpponen et al. 1999b, Walker et al. 2006). Shifts in safe site preferences likely 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* spp. and *Lupinus* spp. at Lyman Glacier forefront, have been argued to be particularly important because they reduce the 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 (vascular plant checklist is available as Supplemental Table S1) in clusters of four 0.25m$^2$ sub-plots located at 20m intervals along four equidistant transects for a total of 228 1m$^2$ sampling units. From these data we calculated plant species richness ($S$), Shannon-Wiener diversity ($H'$), and evenness ($-H'/\ln S$) 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 in del Moral (2002), and regressed that 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), i.e., whether the community PS would decrease or increase over time since deglaciation.

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 years since deglaciation, suggesting a limited control of establishment by the mycorrhizal habit (compare with Read 1991) and limited dependence on presence of mycorrhizal fungus propagules (see also Collier & Bidartondo 2009, Reeves et al. 1979). Overall, the species richness in our sampling limited to a 1m$^2$ scale was low, but comparable to that observed at Coleman Glacier (Jones & del Moral 2005b). At Lyman Glacier, richness reached a maximum of 6.25 ± 0.82 (mean ± 1 st dev) near the terminal moraine. The most species recorded in any one plot was ten.
As common in early primary succession (Matthews 1992, Reiners et al. 1971), plant species richness and diversity increased with time since deglaciation as evidenced by our linear regression analyses (Fig. 2a, 2b). 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 the increasing plant richness and biomass are common observations in chronosequence studies (Matthews 1992, Reiners et al. 1971), our observations contrast with those reported in similar scale for Coleman Glacier (Jones & del Moral 2005b): no dense canopies have established at Lyman Glacier forefront in the first hundred years since glacier retreat.

To classify plant community types (CTs), the 228 experimental units were assigned into seven clusters by complete linkage clustering (JMP version 7.01, SAS Institute, Cary North Carolina) similarly to del Moral (2002), so that clusters retained ~75% of the information on the plot level (Supplemental Figure S1). The seven CTs were characterized by two species that were most strongly associated with that cluster (see insert in Fig. 1).

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 + 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 Luetkea pectinata and the ErM plants Cassiope mertensiana + Phyllodoce empetriformis. None of the EcM Pinaceae (Abies lasiocarpa, Larix lyalii, + 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 comprised of *Luzula piperi* + *Salix phylicifolia*, *Veronica wormskjoldii* + *Pedicularis groenlandica*, and *Pedicularis groenlandica* + *Carex scopularis*, respectively, occur mainly in depressions and near creeks that likely maintain high soil moisture. This emphasizes the difficulties of applying the space-for-time chronosequence approach (Walker *et al.* 2010), but simultaneously indicates the sensitivity of CT analyses to primary determinants 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 homogenous 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 successional communities, the increasing standard deviations suggest a strong contribution of stochastic processes or priority effects during nearly 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 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 at these stages of succession.

Fungal community dynamics

Sources and distribution of fungal propagules

Establishment of fungal propagule banks from atmospheric sources in newly exposed substrates is little debated, but 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 the microbial propagules are everywhere and 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 there are 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: 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 by lichens.

A propagule rain falls on the glacier and its forefront during wind and rain storms, particularly when epigeous, i.e. above-ground fruiting mushrooms, 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, pines and birches establish and persist at least a year after germination without EcM formation, in effect waiting for the EcM inoculum to arrive. This is not likely the case in the Lyman forefront: all Pinaceae sampled, even 1st year seedlings, were colonized by EcM fungi (Cázares et al. 2005). The two systems differ fundamentally: Collier’s & Bidartondo’s (2009) heathland systems have high soil organic matter that provides water retention and nutrients. In contrast, the periglacial outwash at Lyman has little organic matter to sustain seedlings while they wait for EcM colonization to establish. These phenomena are inferred
from few seedling samples, because depletion of seedlings by large-scale 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 commonly fruiting among the EcM trees in 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 with 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 that determines air and water flow likely distribute the propagule pools unevenly across the landscape, thereby establishing safe sites that differ in their spore numbers and compositions.

Although relatively infrequent over the glacier forefront, establishment of EcM willows and conifers and accumulation of leaf litter under their crowns supports fruiting of 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 found on the forefront; only a few of the nearly 70 species in the adjacent subalpine parklands appeared on the forefront (Jumpponen *et al.* 1999a). Several species recorded on the forefront, in return, were not found in 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 locally abundant at both Lyman Glacier and Mt. Fuji. A major propagule source of *I. lacera* and *Cortinarius* and *Laccaria* spp. at Lyman was the old outwash meadow/willow habitat
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 fruited aboveground and the diversity of fungal species gradually increased with plant community development. Based on fruiting body data, this was also true to a degree at Lyman Glacier (Jumpponen et al. 2002): no EcM fruiting bodies were observed up to 300 m from the terminus, two species fruited from 300-400m, and eight from 800-900m. Nara et al. (2003b) found from molecular analysis that several EcM fungi were resupinate; the same is largely true of the Lyman forefront (Trowbridge & Jumpponen 2004). No fruiting bodies of resupinate fungi have been recorded at Lyman (Jumpponen et al. 2002), but spores of hypogeous fungi reliant on animal mycophagy for dispersal were detected on the forefront in 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 mycorrhizae (Cázares & Trappe 1990). Because of its small size (< 3-6mm broad), it could have been more common but overlooked. Nonetheless, 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. Deer are effective in 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, Greipsson & El-Mayas 2000, Koske & Gemma 1997). However, compared to old-field or dune systems, patterns of AM establishment in a montane glacial system may be stochastic, dictated by dispersal by movement of soil that contains inoculum (Warner et
We inferred stochastic AM spore deposition from mycorrhizal colonization, because spores of Glomeromycota were rarely encountered (Cázares et al. 2005). At the Lyman Glacier forefront, dispersal is likely mainly on the feet or in the feces of visiting animals (see also Allen et al. 1984, Warner et al. 1987), with small avalanches or land slides from cliffs and benches that border the forefront, or perhaps in water flow from established plant communities on lateral moraines. These dispersal mechanisms are likely, because AM plants were often more common at the edges than in the center of the forefront. The most recently exposed substrates (15-25 yrs) had 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, likely 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 to those performed for plants, we reanalyzed an EcM dataset (Trowbridge & Jumpponen 2004) and two others on soil communities (Jumpponen 2003, Jumpponen 2007). These data may not compare directly
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 intercanopy environments while some increased in frequencies with time since deglaciation. Trowbridge & Jumpponen (2004) argued 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 with the EcM morphotype data. We analyzed these data with and without including the nonmycorrhizal root tips into the community type (CT) constituents. With nonmycorrhizal tips included, two clusters were identified retained 71.2% of the plot-level variability (not shown) and were defined by two of the three morphotypes assigned to Sordariales in the original analyses (Trowbridge and Jumpponen 2004). Exclusion of the nonmycorrhizal roots revealed five clusters that retained 74.3% of total variability on the plot level (Supplemental 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 rather defined by its heterogeneity than by unifying community components: the common morphotypes (two Sordariales and two Cortinariaceae; Supplemental 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, 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, Jumpponen 2007) to see if we could draw inferences on responses of the fungal communities and/or their components. Like Emerson and Gillespie (2008), our analyses assume immigration and environmental filtering, not speciation, as 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 in del Moral & Jones (2002) and as 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 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, Jumpponen 2007) characterizing soils collected from canopy or intercanopy locations along a Lyman chronosequence. The soil samples originated from 0-900m from glacier terminus and included fifteen from underneath *Salix* canopies (Jumpponen 2007) and seventeen intercanopy samples 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 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 time since deglaciation and canopy condition main effects and their interaction. In this model, Axis 2 PCoA scores increased with time since deglaciation ($F_{3,28} = 4.829, P = 0.0364$) 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 trajectory. In contrast, we found no evidence for canopy effects on community composition. Analyses of standard deviations of PCoA axis scores similar to those we used for plant communities indicated that Axis 3 score standard deviations decrease with distance from glacier terminus ($F_{3,28} = 9.1490, P = 0.0002$) 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 organisms may be enriched in the late primary successional soils. Our earlier studies indicate that EcM fruiting 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 increasing with time since deglaciation included taxa with various non-symbiotic life history strategies: 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 ($F_{1,28} = 0.0068, P = 0.93$) so 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 plant establishment homogenizes soil fungal communities. Although the results of our PCoA and NJ analyses are incongruent, a simple explanation 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 selection of fungi from the local propagule pool based on their compatibility with the *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 microorganisms from stochastic propagule pools.

These conclusions parallel results from a replicated soil transfer 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 Pinus 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 (2 soil types x 2 locations) but 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.

**Successional trajectories of plant and microbial communities compared**

Our synthesis of the work conducted on Lyman Glacier forefront over more than two decades identified research 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 the 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 ‘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 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 that 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 lust for further analyses of microbial community trajectories as well as for patterns and mechanisms of bacterial and fungal 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 into those preceding establishment of plants (Schmidt et al. 2008) and comprised of unexpected and/or novel community constituents (Freeman et al. 2009, Nemergut et al. 2007). Our analyses here and those published elsewhere evidence that successional processes of microbial communities are unlikely to be successfully modeled after those of plants, albeit
the plant controls of 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: the field is wide open for research, especially with newly available molecular and statistical techniques.

Acknowledgements

The research at Lyman Glacier forefront has been supported by US National Science Foundation Grants DEB-9310006 (JMT), DEB-0344838 (AJ), DEB-0516456 (AJ) OPP-0221489 (AJ), National Science Foundation EPSCoR Grant 9874732 with matching support from the State of Kansas (AJ), Emil Aaltonen’s Foundation (AJ), and the US Forest Service Pacific Northwest Research Station. Shawn Brown is supported by Department of Education GAANN program. We are grateful for the abiding logistical support of Ken Dull and Al Murphy of the US Forest Service, Wenatchee National Forest, Chelan Ranger District, Washington. Sid Burns of Domke Lake undertook the challenge of horse-packing research equipment and camp supplies to the base camp at lower Lyman Lake. We thank the many colleagues and friends who participated in the research: Ankie Camacho, Gro Gulden, Leena Hämet-Ahti, Kim Mattson, Nick Otting, and Henry Väre for their help with identification of vascular plants and fungi; Jennifer Anderson, Chad Fox, Rob Dunn, Kei Fujimura, Francesco Gentili, Nicolo Gentili, Lisa Grubisha, Erik Hobbie, Anna Jumpponen, Ashlyn Jumpponen, Kim Mattson, Michael Morneau, Nick Otting, Jamie Platt, Amanda Riffel, and Matt Trappe, who assisted in collecting the many datasets used in this work.

References


Figure legends

**Fig. 1.** Plant community type (CT) frequency dynamics along the 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 Supplemental 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.

**Fig. 2.** Plant community dynamics as a function of the substrate exposure (time since deglaciation) 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 $P > 0.05$; * $P \leq 0.05$; ** $0.01 \leq P < 0.05$; *** $\leq 0.001$. 
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. ns $P > 0.05$; * $P \leq 0.05$; ** $0.01 \leq P < 0.05$; *** $\leq 0.001$.

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. ns $P > 0.05$; * $P \leq 0.05$; ** $0.01 \leq P < 0.05$; *** $\leq 0.001$.

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 shown in Fig. 4. ns $P > 0.05$; * $P \leq 0.05$; ** $0.01 \leq P < 0.05$; *** $\leq 0.001$.

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. ns $P > 0.05$; * $P \leq 0.05$; ** $0.01 \leq P < 0.05$; *** $\leq 0.001$.

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. ns $P > 0.05$; * $P \leq 0.05$; ** $0.01 \leq P < 0.05$; *** $\leq 0.001$.

Fig. 8. Neighbor Joining (NJ) distances (Mean ± Standard Deviation) on a 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. ns $P > 0.05$; * $P \leq 0.05$; ** $0.01 \leq P < 0.05$; *** $\leq 0.001$.

Supplemental Materials

Supplemental Figure S1. Two-way clustering of 228 1m$^2$ characterize plant community types (CTs). Most commonly occurring plant species on the horizontal axis, plots themselves on the vertical axis. The plots were clustered into seven CTs so that approximately 75% of the plot level variability was retained and the CTs characterized by the two most dominant species in each of the seven CTs (see Fig. 1).
**Supplemental Figure S2.** Nonmetric Multidimensional Scaling (NMS) of the plant communities types (CTs) on the forefront of Lyman Glacier. CTs are identified by their respective numbers and their dominant constituent species can be found in the inset of Fig. 1. a) NMS of the first two axes. Following the CT identification, the lower-case letter indicates differences along Axis 1, the upper case letter differences along Axis 2 based on Tukey’s Honestly Significant Difference (HSD) test at \( \alpha = 0.05 \). b) NMS of Axes 1 and 3. Following the CT identification, the lower-case letter indicates differences along Axis 1, the upper case letter differences along Axis 3 based on Tukey’s Honestly Significant Difference (HSD) test at \( \alpha = 0.05 \). Note that CTs 1 and 4 were distinct from other CTs on the first axis, CTs 2 and 7 were separated on the third axis, CT3 was distinct from CTs 6 and 7 on the third axis, and CT3 was distinguished from CTs 4 and 6 on the second axis.

**Supplemental Figure S3.** Two-way clustering to characterize fungal community types (CTs). Most commonly occurring fungal species on the horizontal axis, samples themselves on the vertical axis. The plots were clustered into five CTs so that approximately 75% of the sample level variability was retained.

**Supplemental Table S1.** Checklist of vascular plants recorded at Lyman Glacier Forefront in the North Cascades Mountains of Washington State, U.S.A.