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

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17 *Keywords:* community assembly, community convergence, community divergence,
18 community trajectory, establishment, glacier forefront, mycorrhizae, propagule

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20

21 **ABSTRACT**

22

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

41

42

43 **Introduction**

44

45 Many alpine glaciers reached their glacial maximum during the Little Ice Age in the mid-
46 19th century (Egli *et al.* 2001) and have been retreating over the past century and a half at
47 increasing rates (Dyurgerov & Meier 2000, Hodge *et al.* 1998, Pelto 2006). Glacial retreat
48 exposes a mineral substrate void of organic legacies and often deficient in mineral nitrogen
49 (Matthews 1992, Strauss *et al.* 2009, Tscherko *et al.* 2003), factors that globally limit
50 ecosystem productivity (Vitousek *et al.* 1997). These nutrient limitations are often
51 combined with extreme fluctuations of daily temperatures, limited barriers against wind
52 damage, poor water retention, and high irradiation, all of which may limit plant
53 establishment and survival (Jones & del Moral 2009, Jumpponen *et al.* 1999b, Lichter 2000,
54 Stocklin & Baumler 1996). Additionally, glaciers and their forefronts most often occur at
55 high latitudes and altitudes characterized by short growing seasons and substantial snow
56 cover during the winters. Together, these abiotic stressors make the primary successional
57 forefronts challenging environments but also provide unique opportunities to study early
58 assembly of communities (Cázares *et al.* 2005).

59

60 Many mechanisms controlling primary succession in plant communities have been recently
61 clarified (Pickett *et al.* 2009, Walker & del Moral 2003) and older theories (Clements 1916,
62 Connell & Slatyer 1977) reevaluated. Some of these insights have shifted views on
63 community assembly processes in terrestrial ecosystems. For example, early successional
64 community development seems not to depend on deterministic colonization by pioneering
65 species but rather combines stochastic dispersal and establishment controls (del Moral
66 2009, Fastie 1995) that later are amended by deterministic processes such as biotic
67 competitive and facilitative controls (del Moral 2009).

68

69 Long-term studies in glacier forefronts are rare because of their remote locations and short
70 accessible seasons dictated by high altitudes and latitudes. Instead, glacier forefronts have

71 often been subjected to a chronosequence approach (space-for-time substitution), in which
72 distance from the glacier terminus is considered as a proxy for time since exposure
73 (Cázares *et al.* 2005, Pickett 1989, Walker *et al.* 2010). While suffering from potential
74 correlations between position in the chronosequence and substrate chemistry, fluctuations
75 in weather or climatic conditions, distance to propagule sources, or other environmental
76 parameters (Fastie 1995, Walker *et al.* 2010), the forefronts benefit from providing a single
77 location wherein substrates of different ages can be observed in a relatively homogenous
78 environment (Cázares *et al.* 2005, Jumpponen *et al.* 1998, Matthews 1992, Raffl *et al.* 2006).

79

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

99

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

115

116 While glacier systems differ in numerous characteristics and the conclusions may be
117 context dependent, many general trends and patterns have proved consistent (Orwin *et al.*
118 2006, Tscherko *et al.* 2003). Here, we use Lyman Glacier forefront and our research
119 spanning more than twenty years as a model. We first briefly review patterns of plant
120 community establishment and trajectories, then compare plant and microbial communities.
121 Much of our earlier work focused on mycorrhizal fungus communities; more recent studies
122 broadly emphasize bacterial and fungal communities. Finally, from these cross-domain
123 comparisons, we identify critical areas that have received little attention and propose
124 approaches to address them in fungal and/or microbial systems.

125

126 **Community assembly and ecological filtering**

127

128 As a general framework, we rely on assembly rules used in community ecology (Cole 1983,
129 Hunt 1991). This community assembly model integrates traits and life histories and their
130 contribution to organismal environmental tolerances (Jumpponen & Egerton-Warburton
131 2005). Factors determining successful establishment are considered as abiotic and biotic
132 filters that select community components from local, regional, and ecologically suited
133 species pools (Booth & Swanton 2002, Weiher & Keddy 1995, Weiher & Keddy 2001). Local
134 and regional propagule pools determine candidate species with potential for being
135 included in the community, but the ecological filters (Weiher & Keddy 1995, Weiher &
136 Keddy 2001) remove candidates that fail to establish or persist under the present local
137 environmental (Grubb 1977, Southwood 1988). Combined, the assembly rules outline
138 constraints on selection of communities from larger potential constituent species pools
139 (Weiher & Keddy 2001). These assembly rules can also elucidate processes that produce
140 communities present in a habitat or environment (Booth & Swanton 2002, Drake *et al.*
141 1993). In conclusion, assembly rules and ecological filtering are particularly useful in
142 successional ecology, because they account for both stochastic (*e.g.*, distribution of suitable
143 establishment sites and random distribution of propagules in seed and spore banks) and
144 deterministic factors (*e.g.*, facilitative and competitive processes once communities
145 establish) in a unified framework.

146

147 **Plant community dynamics**

148

149 *Plant establishment*

150

151 Microsites (safe sites sensu Harper *et al.* 1961) where plants establish in primary
152 succession are not random (Jumpponen *et al.* 1999b) but characterized as assemblages
153 with low levels of organization (Robbins & Matthews 2009) where the importance of biotic
154 and abiotic controls shifts over time (del Moral 2009). As a result of environmental
155 heterogeneity, some microsites trap larger propagule numbers or may be more favourable

156 for germination and seedling establishment (Harper *et al.* 1965, Jumpponen *et al.* 1999b,
157 Oswald & Neuenschwander 1993, Titus & del Moral 1998).

158

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

171

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

184

185 *Patterns of plant community development*

186

187 To describe plant community diversity, and heterogeneity at Lyman Glacier, we recorded
188 plant species (vascular plant checklist is available as Supplemental Table S1) in clusters of
189 four 0.25m² sub-plots located at 20m intervals along four equidistant transects for a total
190 of 228 1m² sampling units. From these data we calculated plant species richness (S),
191 Shannon-Wiener diversity (H'), and evenness ($-H'/\ln S$) and compared community
192 compositions using Nonmetric Multidimensional Scaling (NMS - McCune & Grace 2002). To
193 construct a metric for community heterogeneity, we estimated similarity of community
194 compositions among plots, using percent similarity (PS) as described in del Moral (2002),
195 and regressed that against distance from the glacier terminus to test whether or not our
196 communities tended to converge or diverge over time (del Moral & Jones 2002, del Moral
197 2009), *i.e.*, whether the community PS would decrease or increase over time since
198 deglaciation.

199

200 In contrast to Coleman Glacier on Mount Baker, also in Washington state (Jones & del Moral
201 2005b), the most recently deglaciated plots at Lyman Glacier were devoid of vegetation,
202 and nonvegetated plots occurred even near the terminal moraine (Fig. 1). The first
203 individuals of the nonmycorrhizal *Luzula piperi* and *Saxifraga ferruginea*, and the
204 ectomycorrhizal *Abies lasiocarpa* had established after approximately 15 years since
205 deglaciation, suggesting a limited control of establishment by the mycorrhizal habit
206 (compare with Read 1991) and limited dependence on presence of mycorrhizal fungus
207 propagules (see also Collier & Bidartondo 2009, Reeves *et al.* 1979). Overall, the species
208 richness in our sampling limited to a 1m² scale was low, but comparable to that observed at
209 Coleman Glacier (Jones & del Moral 2005b). At Lyman Glacier, richness reached a
210 maximum of 6.25 ± 0.82 (mean \pm 1 st dev) near the terminal moraine. The most species
211 recorded in any one plot was ten.

212

213 As common in early primary succession (Matthews 1992, Reiners *et al.* 1971), plant species
214 richness and diversity increased with time since deglaciation as evidenced by our linear
215 regression analyses (Fig. 2a, 2b). Evenness was low (0.23 ± 0.08) and constant with time
216 since deglaciation. These patterns stem from sparse plant occurrence near the glacier
217 terminus and increasing but still heterogeneous cover near the terminal moraine. Although
218 the increasing plant richness and biomass are common observations in chronosequence
219 studies (Matthews 1992, Reiners *et al.* 1971), our observations contrast with those
220 reported in similar scale for Coleman Glacier (Jones & del Moral 2005b): no dense canopies
221 have established at Lyman Glacier forefront in the first hundred years since glacier retreat.

222

223 To classify plant community types (CTs), the 228 experimental units were assigned into
224 seven clusters by complete linkage clustering (JMP version 7.01, SAS Institute, Cary North
225 Carolina) similarly to del Moral (2002), so that clusters retained ~75% of the information
226 on the plot level (Supplemental Figure S1). The seven CTs were characterized by two
227 species that were most strongly associated with that cluster (see insert in Fig. 1).
228 Evaluation of the CT frequencies along the Lyman Glacier forefront permits an assessment
229 of community trajectories and their dynamics. Nonvegetated patches and early establishing
230 nonmycorrhizal plants (CTs 1 and 4, with *Juncus drummondii* + *Saxifraga ferrugina*) occur
231 commonly but in variable frequencies throughout the forefront. The common communities
232 near the terminal moraine are CTs 2 and 7, comprised primarily of plant species frequent
233 in the montane parkland habitat outside the forefront, including the AM-forming *Luetkea*
234 *pectinata* and the ErM plants *Cassiope mertensiana* + *Phyllodoce empetriformis*. None of the
235 EcM Pinaceae (*Abies lasiocarpa*, *Larix lyalii*, + *Tsuga mertensiana*), common in forested
236 patches among the alpine meadows adjacent to the forefront, are frequent enough to weigh
237 in the community assignments.

238

239 These analyses suggest that, not only the successional trajectories, but also the
240 environmental heterogeneity in this system dictate the CT occurrence. To exemplify, CTs 3,
241 5 and 6 comprised of *Luzula piperi* + *Salix phylicifolia*, *Veronica wormskjoldii* + *Pedicularis*
242 *groenlandica*, and *Pedicularis groenlandica* + *Carex scopularis*, respectively, occur mainly in
243 depressions and near creeks that likely maintain high soil moisture. This emphasizes the
244 difficulties of applying the space-for-time chronosequence approach (Walker *et al.* 2010),
245 but simultaneously indicates the sensitivity of CT analyses to primary determinants of
246 plant community development.

247

248 *Deterministic vs. stochastic processes in the plant community succession*

249

250 Plant community convergence towards a terminal (climax) state community was integral in
251 early concepts of succession (Clements 1916). If the deterministic processes were to
252 increase with succession, then plant community composition should follow a predictable
253 trajectory (del Moral 2009) and become more homogenous over successional time (Leps &
254 Rejmanek 1991). However, convergence towards a stable community (Pickett 1989) may
255 depend on factors such as heterogeneity of the local species or their propagule pools,
256 strength of the biological legacies, importance of priority effects, and sensitivity to
257 conditions during initial stages of succession (Walker *et al.* 2010). We discuss below
258 trajectories based on ordination analyses and changes in community similarities among
259 plots along the chronosequence.

260

261 In our plant community ordination analyses, NMS scores for the first axis increased
262 linearly, suggesting a predictable trajectory along this axis (Fig. 3) as suggested in early
263 concepts of succession (Clements 1916). Changes in the other two axes were not linear but
264 were best characterized by regression models that included quadratic terms (data not
265 shown). When standard deviations of NMS axis scores were analyzed by linear regression,
266 they increased linearly for axis 1 (Fig. 4) and axis 2 (not shown). While communities may

267 be changing to include a greater component from surrounding late successional
268 communities, the increasing standard deviations suggest a strong contribution of stochastic
269 processes or priority effects during nearly a century since deglaciation (del Moral 2009).
270 More importantly, the increasing standard deviations suggest that these periglacial plant
271 communities are characterized by community divergence rather than convergence during
272 early succession.

273

274 To further illuminate the determinism in the plant communities, we calculated percent
275 similarity (PS) for each group of plots representing equal time since deglaciation (see del
276 Moral & Jones 2002). In contrast to increasing determinism in the primary successional
277 system at Mount St. Helens in Washington state (del Moral 2009), our PS estimates
278 decreased with time since deglaciation (Fig. 5). This observation corroborates the analyses
279 of the NMS standard deviations and points to stochastic processes. These findings are
280 primarily attributable to the occurrence of a large number of different CTs and the
281 persistence of the early successional communities near the terminal moraine. In sum, we
282 conclude that plant communities at Lyman Glacier site are diverging rather than
283 converging at these stages of succession.

284

285 **Fungal community dynamics**

286

287 *Sources and distribution of fungal propagules*

288

289 Establishment of fungal propagule banks from atmospheric sources in newly exposed
290 substrates is little debated, but distribution of these banks has received little attention. It is
291 uncertain whether microtopological characteristics similar to those for plant safe sites
292 accumulate microbes as a result of reduced air velocities or water flow. The Baas-Becking
293 hypothesis (Martiny *et al.* 2006) states that because of their small size and enormous

294 numbers the microbial propagules are everywhere and environment selects from the
295 established propagule banks. In contrast to such environmentally based niche models,
296 recent work suggests that microbial communities, like those of macroscopic eukaryotes,
297 may suffer from dispersal limitations. They may thus be assembled by stochastic
298 immigration, establishment, and local extinction events (Peay *et al.* 2010).

299

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

309

310 A propagule rain falls on the glacier and its forefront during wind and rain storms,
311 particularly when epigeous, *i.e.* above-ground fruiting mushrooms, release spores.
312 Consequently, propagules of EcM fungi are likely to be available to hosts in early
313 succession. This was implicit in the EcM conifer seedlings appearing early in the primary
314 succession. Collier & Bidartondo (2009) reported on heathlands where EcM spores are
315 rare, pines and birches establish and persist at least a year after germination without EcM
316 formation, in effect waiting for the EcM inoculum to arrive. This is not likely the case in the
317 Lyman forefront: all Pinaceae sampled, even 1st year seedlings, were colonized by EcM
318 fungi (Cázares *et al.* 2005). The two systems differ fundamentally: Collier's & Bidartondo's
319 (2009) heathland systems have high soil organic matter that provides water retention and
320 nutrients. In contrast, the periglacial outwash at Lyman has little organic matter to sustain
321 seedlings while they wait for EcM colonization to establish. These phenomena are inferred

322 from few seedling samples, because depletion of seedlings by large-scale sampling would
323 have drastically interfered with early successional stages.

324

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

338

339 Although relatively infrequent over the glacier forefront, establishment of EcM willows and
340 conifers and accumulation of leaf litter under their crowns supports fruiting of EcM fungi.
341 Repeated searches over more than a decade on the forefront revealed that in all,
342 sporocarps of only 13 species of EcM fungi were found on the forefront; only a few of the
343 nearly 70 species in the adjacent subalpine parklands appeared on the forefront
344 (Jumpponen *et al.* 1999a). Several species recorded on the forefront, in return, were not
345 found in surrounding areas (Jumpponen *et al.* 2002). The most abundant genera were
346 *Cortinarius*, *Inocybe* and *Laccaria*. These genera were also common in primary successional,
347 upper montane and subalpine habitats under *Salix* on volcanic substrates on Mt. Fuji, Japan
348 (Nara *et al.* 2003a). Two species, *Cortinarius decipiens* and *Inocybe lacera*, were observed
349 locally abundant at both Lyman Glacier and Mt. Fuji. A major propagule source of *I. lacera*
350 and *Cortinarius* and *Laccaria* spp. at Lyman was the old outwash meadow/willow habitat

351 probably several thousand years old immediately below the terminal moraine (J. Trappe,
352 unpublished data).

353

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

374

375 AM fungi increase steadily after disturbance in many early successional ecosystems,
376 indicating successional dynamics of these communities (Allen & Allen 1980, Gemma &
377 Koske 1990, Greipsson & El-Mayas 2000, Koske & Gemma 1997). However, compared to
378 old-field or dune systems, patterns of AM establishment in a montane glacial system may
379 be stochastic, dictated by dispersal by movement of soil that contains inoculum (Warner *et*

380 *al.* 1987). We inferred stochastic AM spore deposition from mycorrhizal colonization,
381 because spores of Glomeromycota were rarely encountered (Cázares *et al.* 2005). At the
382 Lyman Glacier forefront, dispersal is likely mainly on the feet or in the feces of visiting
383 animals (see also Allen *et al.* 1984, Warner *et al.* 1987), with small avalanches or land slides
384 from cliffs and benches that border the forefront, or perhaps in water flow from
385 established plant communities on lateral moraines. These dispersal mechanisms are likely,
386 because AM plants were often more common at the edges than in the center of the
387 forefront. The most recently exposed substrates (15-25 yrs) had no or few AM plants, but
388 otherwise no clear patterns were evident, further evidencing the stochastic nature of AM
389 spore dispersal. Helm *et al.* (1996) reported that AM colonization and spores were
390 infrequent and showed no particular patterns on the forefront of an Alaskan low-elevation
391 glacier, further evidencing spore dispersal limitation in these habitats.

392

393 Our analyses above show that CTs with *Cassiope* and *Phyllodoce* increase late on the Lyman
394 Glacier chronosequence. While arrival of the ErM fungi could not be observed directly, ErM
395 colonization of the susceptible hosts gradually increased over time since soil exposure
396 (Cázares *et al.* 2005), suggesting gradual buildup of an inoculum pool, likely from air-
397 and/or ungulate-borne propagules. Similarly to ErM, dark septate endophyte (DSE)
398 colonization gradually increased over the chronosequence regardless of the mycorrhizal
399 habit of the host (Cázares *et al.* 2005). These observations parallel those of Peay *et al.*
400 (2010): dispersal seems a key control of fungal community composition in early
401 successional stages.

402

403 *Patterns of fungal community development*

404

405 To attempt fungal community analyses comparable to those performed for plants, we
406 reanalyzed an EcM dataset (Trowbridge & Jumpponen 2004) and two others on soil
407 communities (Jumpponen 2003, Jumpponen 2007). These data may not compare directly

408 with those for plants because of differences in data volumes and recording. However, they
409 illustrate contrasts and similarities between fungal and plant communities and identify
410 further research needs.

411

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

422

423 To compare successional trajectories between fungal and plant communities, we used
424 complete linkage clustering with the EcM morphotype data. We analyzed these data with
425 and without including the nonmycorrhizal root tips into the community type (CT)
426 constituents. With nonmycorrhizal tips included, two clusters were identified retained
427 71.2% of the plot-level variability (not shown) and were defined by two of the three
428 morphotypes assigned to Sordariales in the original analyses (Trowbridge and Jumpponen
429 2004). Exclusion of the nonmycorrhizal roots revealed five clusters that retained 74.3% of
430 total variability on the plot level (Supplemental Fig. S3). These community analyses were
431 uninformative with respect to time since deglaciation or canopy environment because
432 three of the five clusters occurred only once, twice, and three times in the 30 samples. The
433 most common cluster, occurring 19 times, was rather defined by its heterogeneity than by
434 unifying community components: the common morphotypes (two Sordariales and two
435 Cortinariaceae; Supplemental Fig. S3) were common constituents of this CT.

436

437 These analyses highlight fundamental issues that complicate fungal community analyses. A
438 ten-fold increase in sampling, similar to that for plant communities, would be difficult to
439 achieve by microscopic EcM morphotyping and molecular identification. Moreover, while
440 the EcM communities may not be as diverse as those in soil (Buée *et al.* 2009), their
441 complete characterization even in an early successional system with limited diversity
442 would be difficult. However, development of high throughput parallel sequencing tools
443 (Cardenas & Tiedje 2008) combined with DNA-tagging (Meyer *et al.* 2008) might solve the
444 issues necessary to differentiate among the root-inhabiting fungal communities.

445

446 *Deterministic vs. stochastic processes in fungal community succession*

447

448 We analyzed combined datasets (from Jumpponen 2003, Jumpponen 2007) to see if we
449 could draw inferences on responses of the fungal communities and/or their components.
450 Like Emerson and Gillespie (2008), our analyses assume immigration and environmental
451 filtering, not speciation, as the fundamental processes of community assembly in early
452 succession. The scale of a glacier forefront is unlikely to lead to cladogenic speciation,
453 mainly because the large allochthonous source populations maintain geneflow into the
454 periglacial environment.

455

456 To test whether the EcM communities associated with *Salix* were undergoing community-
457 level convergence or divergence, we analyzed the NMS axis score standard deviations and
458 estimated PS as described in del Moral & Jones (2002) and as above for plant communities.
459 When the standard deviations of the NMS axis scores were analyzed by linear regression
460 with vs without the terms that would account for the canopy environment plus interaction
461 terms, the axis score standard deviations showed no significant effects of either time since
462 deglaciation or canopy ($P > 0.15$ for all effect tests using ANOVA or t-tests for null

463 hypotheses that parameter estimates equal zero). Similarly, none of the simple linear or
464 multiple regression models analyzed to test effects of *Salix* canopies and time since
465 deglaciation on PS differed significantly for convergence or divergence ($P > 0.30$ for all
466 effect tests using ANOVA or t-tests for null hypotheses that parameter estimates equal
467 zero). In other words, we found no support for patterns similar to those observed for plant
468 communities.

469

470 We also targeted general fungal communities associated with soils underneath *Salix*
471 canopies vs. intercanopy areas using an approach similar to that described above. In these
472 analyses, we tested whether differential phylogenetic clustering between the two canopy
473 environments could be detected or whether phylogenetic patterns could be visualized in
474 relation to time since deglaciation. Using the data matrices comparing within-sample
475 phylogenetic distances among the experimental units, we sought stochastic vs.
476 deterministic trajectories of fungal communities. To do this, we re-analyzed Small Sub-Unit
477 (SSU) sequences of the ribosomal RNA gene from two clone library studies (Jumpponen
478 2003, Jumpponen 2007) characterizing soils collected from canopy or intercanopy
479 locations along a Lyman chronosequence. The soil samples originated from 0-900m from
480 glacier terminus and included fifteen from underneath *Salix* canopies (Jumpponen 2007)
481 and seventeen intercanopy samples across the chronosequence (Jumpponen 2003). We
482 reanalyzed representative sequences from the two studies and MUSCLE-aligned them using
483 Geneious Pro 5.3.4 (Biomatters Ltd., Chirstchurch, New Zealand). The alignments were
484 analyzed by neighbor joining (NJ) method and the tree distance matrix obtained was
485 analyzed with Fast Unifrac (Hamady *et al.* 2010) with a Principal Coordinates Analysis
486 (PCoA). The PCoA scores obtained for the first three axes (representing 20.6%, 11.0%, and
487 7.1% of the variability) and the NJ distance matrix were used to analyze community
488 similarity described above by use of both the standard deviations of PCoA scores and
489 within-sample mean NJ distances to test hypotheses on community convergence or
490 divergence.

491

492 Analyses of PCoA axis scores indicated that Axis 1 and Axis 3 scores did not vary
493 significantly with time since deglaciation or canopy condition. In contrast, Axis 2 seemed to
494 drive the observed patterns in a full model (goodness of fit; $F_{3,28}=6.4361$, $P=0.0019$) with
495 time since deglaciation and canopy condition main effects and their interaction. In this
496 model, Axis 2 PCoA scores increased with time since deglaciation (Fig. 6; $F_{3,28} = 4.829$, $P =$
497 0.0364) indicating a trajectory in fungal community composition based on SSU sequence
498 data. We interpret these data to indicate that the communities are shifting somewhat
499 predictably and incorporating different community members to lead to such trajectory. In
500 contrast, we found no evidence for canopy effects on community composition. Analyses of
501 standard deviations of PCoA axis scores similar to those we used for plant communities
502 indicated that Axis 3 score standard deviations decrease with distance from glacier
503 terminus (Fig. 7; full model goodness of fit $F_{3,28} = 9.1490$, $P = 0.0002$) suggesting
504 phylogenetic convergence with time since deglaciation. However, standard deviations for
505 Axis 1 and 2 scores showed no response to time since deglaciation, suggesting minor
506 convergent patterns.

507

508 These analyses of the PCoA scores and their standard deviations provide a starting point
509 for asking which organisms may be enriched in the late primary successional soils. Our
510 earlier studies indicate that EcM fruiting bodies (Jumpponen *et al.* 2002), root-associated
511 propagules (Trowbridge & Jumpponen 2004), and the root colonization of various hosts
512 (Cázares *et al.* 2005) increase over successional time. However, only *Tomentella*
513 (Thelephoraceae; GenBank Accession DQ092920) increased in frequency with time since
514 deglaciation and none decreased. Others also increasing with time since deglaciation
515 included taxa with various non-symbiotic life history strategies: *Mycoacia* (Merulinaceae;
516 DQ873636) and *Pulvinula* (Pyronemataceae; U62012). Thus, the increasing trajectory of
517 Axis 2 PCoA scores and convergence indicated by declining Axis 3 PCoA score standard
518 deviations corroborate results of our earlier studies and evidence a relationship of plant
519 establishment with subsequent fungal community enrichment.

520

521 In addition to PCoA, we analyzed the SSU RNA gene NJ distance matrix to directly compare
522 sample-level distances in *Salix* canopy and intercanopy soils along the forefront
523 chronosequence. These analyses tested if these communities show a random assembly
524 from the regional propagule pools and would therefore possess equal ability to establish
525 across the periglacial substrates. Alternatively, if environmental filters shape the
526 establishing communities, the communities should show phylogenetic structuring –
527 perhaps related to the establishment of few organisms that share similar preferences for
528 habitat or hosts but are not competitively exclusive. In contrast to the analyses of Axis 2
529 PCoA scores, fungal community NJ distances did not vary with time since deglaciation ($F_{1,28}$
530 = 0.0068, $P = 0.93$) so the fungal communities are not strongly affected by time of substrate
531 exposure from underneath the glacier (Fig. 8). However, judging from these analyses,
532 fungal communities of canopy soils had significantly lower NJ distances than those from
533 non-vegetated areas (Fig. 8; $F_{2,28} = 11.1$, $P = 0.0022$). The nonsignificant interaction term
534 indicates canopy effects regardless of position in the periglacial chronosequence. We
535 conclude that fungal communities converge in the canopy soils relative to nonvegetated
536 soils and plant establishment homogenizes soil fungal communities. Although the results of
537 our PCoA and NJ analyses are incongruent, a simple explanation that, while the intercanopy
538 communities may be a random draw of propagules, the canopy soils in the sparsely-
539 vegetated early plant communities are enriched for fungi that rely on host photosynthates
540 or litter. To better tie this to the community assembly model that we introduced previously
541 (Jumpponen & Egerton-Warburton 2005), this convergence can be argued to result from
542 selection of fungi from the local propagule pool based on their compatibility with the *Salix*
543 and its canopy soil environment. This is best exemplified by foliage-associated *Coniochaeta*
544 (GenBank Accession GQ154624), the only taxon positively associated with canopies: it did
545 not respond to time since deglaciation in our analyses.

546

547 The convergence inferred from the NJ distances corroborates our earlier PLFA studies
548 (Ohtonen *et al.* 1999), in which microbial communities differed between canopy and
549 intercanopy soils. These results emphasize the importance of plant establishment in
550 shaping early microbial communities (Ohtonen *et al.* 1999). While compositional changes

551 with time since deglaciation were not obvious, the samples collected underneath
552 established plants were more tightly clustered in the NMS ordination than those collected
553 from nonvegetated areas (see Fig. 2 in Ohtonen *et al.* 1999). Although those data do not
554 permit a more thorough convergence analysis, we propose that, in combination with our NJ
555 distance analyses, plants tend to homogenize microbial communities and select subsets of
556 compatible microorganisms from stochastic propagule pools.

557

558 These conclusions parallel results from a replicated soil transfer on the Lyman forefront
559 (Jumpponen *et al.* 1998). In those studies, small mesh bags were filled with willow canopy
560 soil and an equal number with intercanopy soils. Seeds of *Pinus contorta*, which occurs on
561 the forefront, were planted in each bag. Separate holes to accommodate one bag of each
562 soil type were dug under canopies and in intercanopy sites. Seedling emergence and
563 survival were recorded 8 weeks after sowing and were low in all treatments (2 soil types x
564 2 locations) but on average twice as high in the canopy soil transferred to intercanopy
565 spaces than in other treatments. Canopies thus appeared to suppress emergence/survival
566 and intercanopy soils were similarly adverse in that respect. The superior performance of
567 seedlings in canopy soil in intercanopy sites indicates that the combined enhancement of
568 nutrients and microbes provided greater benefit when the suppressive effects of the
569 canopies themselves were removed.

570

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

572

573 Our synthesis of the work conducted on Lyman Glacier forefront over more than two
574 decades identified research gaps in our current understanding of the fundamental
575 processes of early microbial community assembly. Early propagule accumulation and the
576 processes that control it prior to the community assembly are poorly understood for both
577 microbial and plant communities. A framework of safe microsites for plant seed
578 accumulation and seedling establishment exists, but the presence of such sites for

579 microbial communities remains uncertain. Mechanisms that control selection of active
580 microbial community members from established propagule banks are equally unclear. The
581 difficulty of selecting an appropriate scale for 'microbial landscape' and its ecology
582 complicates these issues.

583

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

597

598 We hope that these analyses and discussions will arouse lust for further analyses of
599 microbial community trajectories as well as for patterns and mechanisms of bacterial and
600 fungal community divergence and convergence. Microbial communities tend to be orders of
601 magnitude more complex than those of plants and animals, but the extreme environments
602 in primary successional ecosystems limit microbial richness. Furthermore, recent studies
603 provide insights into microbial ecosystem functions and processes, particularly into those
604 preceding establishment of plants (Schmidt *et al.* 2008) and comprised of unexpected
605 and/or novel community constituents (Freeman *et al.* 2009, Nemergut *et al.* 2007). Our
606 analyses here and those published elsewhere evidence that successional processes of
607 microbial communities are unlikely to be successfully modeled after those of plants, albeit

608 the plant controls of microbial communities must be accounted for after plant
609 establishment. We conclude that microbial communities and their dynamics express novel
610 community level processes with important consequences for plant community
611 development: the field is wide open for research, especially with newly available molecular
612 and statistical techniques.

613

614 **Acknowledgements**

615

616 The research at Lyman Glacier forefront has been supported by US National Science
617 Foundation Grants DEB-9310006 (JMT), DEB-0344838 (AJ), DEB-0516456 (AJ) OPP-
618 0221489 (AJ), National Science Foundation EPSCoR Grant 9874732 with matching support
619 from the State of Kansas (AJ), Emil Aaltonen's Foundation (AJ), and the US Forest Service
620 Pacific Northwest Research Station. Shawn Brown is supported by Department of
621 Education GAANN program. We are grateful for the abiding logistical support of Ken Dull
622 and Al Murphy of the US Forest Service, Wenatchee National Forest, Chelan Ranger District,
623 Washington. Sid Burns of Domke Lake undertook the challenge of horse-packing research
624 equipment and camp supplies to the base camp at lower Lyman Lake. We thank the many
625 colleagues and friends who participated in the research: Ankie Camacho, Gro Gulden, Leena
626 Hämet-Ahti, Kim Mattson, Nick Otting, and Henry Väre for their help with identification of
627 vascular plants and fungi; Jennifer Anderson, Chad Fox, Rob Dunn, Kei Fujimura, Francesco
628 Gentili, Nicolo Gentili, Lisa Grubisha, Erik Hobbie, Anna Jumpponen, Ashlyn Jumpponen,
629 Kim Mattson, Michael Morneau, Nick Otting, Jamie Platt, Amanda Riffel, and Matt Trappe,
630 who assisted in collecting the many datasets used in this work.

631

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1000 **Figure legends**

1001

1002 **Fig. 1.** Plant community type (CT) frequency dynamics along the time since substrate
1003 exposure in the forefront of the receding Lyman Glacier. The inset identifies the dominant
1004 components of the CTs based on two-way complete linkage clustering shown in
1005 Supplemental Fig. S1. Note that the CT1 – characterized by nonmycorrhizal *Saxifraga* and
1006 nonvegetated, open areas – is frequent through the chronosequence even close to the
1007 terminal moraine.

1008

1009 **Fig. 2.** Plant community dynamics as a function of the substrate exposure (time since
1010 deglaciation) in the forefront of the receding Lyman Glacier: a) plant species richness (S)
1011 increases, the inset identifies the linear regression model with significant intercept and
1012 slope terms; b) Shannon-Wiener diversity (H') increases, the inset identifies the linear
1013 regression model with significant intercept and slope terms. ^{ns} $P > 0.05$; * $P \leq 0.05$; ** $0.01 \leq$
1014 $P < 0.05$; *** ≤ 0.001 .

1015

1016 **Fig. 3.** Mean Nonmetric Multidimensional Scaling (NMS) Axis 1 scores of the plant
1017 community types (CTs) along the Lyman Glacier forefront increase with time since
1018 deglaciation. The inset identifies the linear regression model with significant intercept and
1019 slope terms. The linear increase in the NMS scores suggests a successional trajectory in this
1020 primary successional system and is likely correlated with increasing abundance of the CTs
1021 with component species from the adjacent montane meadow communities. ^{ns} $P > 0.05$; * $P \leq$
1022 0.05 ; ** $0.01 \leq P < 0.05$; *** ≤ 0.001 .

1023

1024 **Fig. 4.** Mean Nonmetric Multidimensional Scaling (NMS) Axis 1 score standard deviations
1025 of the plant community types (CTs) along the Lyman Glacier forefront increase with time
1026 since deglaciation. The inset identifies the linear regression model with a significant slope
1027 term. The increase in the NMS score standard deviations suggests a divergence of the plant
1028 communities in this primary successional system and is likely a result of stochastic plant
1029 establishment processes and heterogeneous distribution of the CTs along the successional
1030 chronosequence. ^{ns} $P > 0.05$; * $P \leq 0.05$; ** $0.01 \leq P < 0.05$; *** ≤ 0.001 .

1031

1032 **Fig. 5.** Percent similarity (PS) of the plant communities along the Lyman Glacier forefront
1033 decline with time since deglaciation, the inset identifies the linear regression model with
1034 significant intercept and slope terms. The decrease in the PS corroborates divergence of the
1035 plant communities in this primary successional system shown in Fig. 4. ^{ns} $P > 0.05$; * $P \leq$
1036 0.05 ; ** $0.01 \leq P < 0.05$; *** ≤ 0.001 .

1037

1038 **Fig. 6.** Principal Coordinates Analysis (PCoA) Axis 2 scores for fungal communities along
1039 the Lyman Glacier forefront increase with time since deglaciation. The inset identifies the
1040 linear regression model with significant terms for intercept and slope but not for canopy
1041 position or interaction. The linear increase in the PCoA scores suggests a successional

1042 trajectory in this primary successional system. ^{ns} $P > 0.05$; * $P \leq 0.05$; ** $0.01 \leq P < 0.05$; ***
1043 ≤ 0.001 .

1044

1045 **Fig. 7.** Principal Coordinates Analysis (PCoA) Axis 3 score standard deviations for fungal
1046 communities along the Lyman Glacier forefront decrease with time since deglaciation. The
1047 inset identifies the linear regression model with significant terms for intercept and slope
1048 but not for canopy position or interaction. The linear decrease in the standard deviations
1049 suggests a community convergence over time in this primary successional system. ^{ns} $P >$
1050 0.05 ; * $P \leq 0.05$; ** $0.01 \leq P < 0.05$; *** ≤ 0.001 .

1051

1052 **Fig. 8.** Neighbor Joining (NJ) distances (Mean \pm Standard Deviation) on a sample level for
1053 fungal communities along the Lyman Glacier forefront are greater in the canopy samples
1054 than in the intercanopy samples. The inset identifies the linear regression model with
1055 significant terms for intercept and canopy position but not for slope or the interaction. The
1056 difference between the canopy and intercanopy samples suggests a canopy soil community
1057 convergence relative to intercanopy soils in this primary successional system. The two
1058 groups of samples with different NJ distances are indicated by P -values (oneway ANOVA)
1059 above the bars. ^{ns} $P > 0.05$; * $P \leq 0.05$; ** $0.01 \leq P < 0.05$; *** ≤ 0.001 .

1060

1061 **Supplemental Materials**

1062

1063 **Supplemental Figure S1.** Two-way clustering of 228 1m² characterize plant community
1064 types (CTs). Most commonly occurring plant species on the horizontal axis, plots
1065 themselves on the vertical axis. The plots were clustered into seven CTs so that
1066 approximately 75% of the plot level variability was retained and the CTs characterized by
1067 the two most dominant species in each of the seven CTs (see Fig. 1).

1068

1069 **Supplemental Figure S2.** Nonmetric Multidimensional Scaling (NMS) of the plant
1070 communities types (CTs) on the forefront of Lyman Glacier. CTs are identified by their
1071 respective numbers and their dominant constituent species can be found in the inset of Fig.
1072 1. a) NMS of the first two axes. Following the CT identification, the lower-case letter
1073 indicates differences along Axis 1, the upper case letter differences along Axis 2 based on
1074 Tukey's Honestly Significant Difference (HSD) test at $\alpha = 0.05$. b) NMS of Axes 1 and 3.
1075 Following the CT identification, the lower-case letter indicates differences along Axis 1, the
1076 upper case letter differences along Axis 3 based on Tukey's Honestly Significant Difference
1077 (HSD) test at $\alpha = 0.05$. Note that CTs 1 and 4 were distinct from other CTs on the first axis,
1078 CTs 2 and 7 were separated on the third axis, CT3 was distinct from CTs 6 and 7 on the
1079 third axis, and CT3 was distinguished from CTs 4 and 6 on the second axis.

1080

1081 **Supplemental Figure S3.** Two-way clustering to characterize fungal community types
1082 (CTs). Most commonly occurring fungal species on the horizontal axis, samples themselves
1083 on the vertical axis. The plots were clustered into five CTs so that approximately 75% of the
1084 sample level variability was retained.

1085

1086 **Supplemental Table S1.** Checklist of vascular plants recorded at Lyman Glacier Forefront
1087 in the North Cascades Mountains of Washington State, U.S.A.

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