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Mycorrhiza-plant colonization patterns on a subalpine glacier forefront as a model system of primary succession

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Abstract Lyman glacier in the North Cascades Mountains of Washington has a subalpine forefront characterized by a well-developed terminal moraine, inconspicuous successional moraines, fluting, and outwash. These deposits were depleted of symbiotic fungi when first exposed but colonized by them over time after exposure. Four major groups of plant species in this system are (1) mycorrhiza-independent or facultative mycotrophic, (2) dependent on arbuscular mycorrhizae (AM) (3) dependent on ericoid mycorrhiza (ERM) or ectomycorrhizae (EM), and (4) colonized by dark-septate (DS) endophytes. We hypothesized that availability of mycorrhizal propagules was related to the success of mycorrhiza-dependent plants in colonizing new substrates in naturally evolved ecosystems. To test this hypothesis roots samples of 66 plant species were examined for mycorrhizal colonization. The plants were sampled from communities at increasing distances from the glacier terminus to compare the newest communities with successively older ones. Long established, secondary successional dry meadow communities adjacent to the glacier forefront, and nearby high alpine communities were sampled for comparison. DS were common on most plant species on the forefront. Nonmycorrhizal plants predominated in the earlier successional sites, whereas the proportion of mycorrhizal plants generally increased with age of community. AM were present, mostly at low levels, and nearly absent in two sites of the forefront. ERM were present in all species of Ericaceae sampled, and EM in all species of Pinaceae and Salicaceae. Roots of plants in the long established meadow and heath communities adjacent to the forefront and the high alpine community all had one or another of the colonization types, with DS and AM predominating.

Keywords Arbuscular mycorrhizae · Ectomycorrhizae · Ericoid mycorrhizae · Dark septate endophytes · Fungal propagules

Introduction

Plants with mycorrhizal associations predominate in most natural ecosystems (Dominik 1961; Trappe 1977; Allen 1991). However, the supply of inoculum may be limited in primary successional ecosystems, such as the forefronts of receding glaciers. The presence of compatible fungal partners may be vital for successful plant establishment and long-term survival in ecosystems with no prior history of vegetation cover. Additionally, these fungi may contribute to the development of the early plant community by creating common mycelial networks for nutrient transport between plant individuals (Read et al. 1985; Newman 1988).

Plant succession studies on new substrates traditionally focus on aboveground biotic observations coupled with climatic, topographic and non-biological edaphic factors. However, mycotrophic patterns of colonizing plants correlate strongly with successional stages of plant communities in disturbed and nondisturbed habitats (Reeves et al. 1979; Allen and Allen 1984; Allen et al. 1987; Trappe 1987; Helm et al. 1996).

Four major groups of plant species can be differentiated in terms of mycorrhizal ecology of primary plant succession in our study: (1) mycorrhiza-independent or facultative mycorrhizal (facultative meaning sometimes forming mycorrhizae but able to survive and reproduce without them), (2) dependent on arbuscular mycorrhizae (AM), (3) dependent on ericoid mycorrhiza (ERM) or ectomycorrhizae (EM), and (4) colonized by dark-septate (DS) endophytes (Jumpponen and Trappe 1998; Trappe and Luoma 1992; Trappe 1988). The first group can establish in the absence of mycobionts. Mycobiont propagules for the

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second, AM-dependent group must be transported to the new substrate either by movement of propagule-bearing soil, because AM fungi most often form single or clustered statismospores in soil among roots of host plants, or on the bodies or in feces of invertebrates or vertebrates. Propagules of the third, EM- or ERM-dependent group may be forcibly discharged from epigeous sporocarps for broadcast by air or, in the case of hypogeous sporocarps, deposited in feces of animal mycophagists (Cázares and Trappe 1994). Dispersal mechanisms of the fourth group, the DS endophytes, are unknown but most likely by air movement.

Alpine zones in general are characterized by a higher proportion of mycorrhiza-independent or facultative species (group 1) than mycorrhiza-dependent species (groups 2–4) (Read and Haselwandter 1981; Currah and Van Dyk 1986; Trappe 1987, 1988). These, plus EM and ERM species (group 2), are common early colonizers, whereas AM hosts (group 3), e.g., most perennial forbs, are infrequent as such (J.M. Trappe, E. Cázares, D. Luoma and T. O'Dell, unpublished data). DS endophytes (group 4) are common root colonizers of many plant species in diverse habitats (Read and Haselwandter 1981; Sengupta et al. 1989; Kohn and Stasovski 1990; Stoyke and Currah 1991; Jumpponen and Trappe 1998).

These groupings lead to the hypothesis that availability of mycorrhizal propagules relates to which plant species can successfully colonize new substrates under natural conditions of plant succession (Trappe 1988; Trappe and Luoma 1992). To test this hypothesis we compared the availability of mycorrhizal propagules in the soil, and mycotrophy of plant species, in the youngest plant communities established near the glacier terminus to that of three older communities occurring at increasing distances from the terminus on the forefront. Plants from a secondary-successional dry meadow community adjacent to the forefront and a nearby high alpine community were also sampled for comparison with the primary successional communities of the forefront. The data can also serve as a baseline for future studies on changes in these communities over time or in response to global warming.

Materials and methods

The study sites

Lyman glacier (48°10′N, 120°54′W) is at the southern head of a U-shaped valley running south to north and bounded by cliffs in the North Cascades Mountains of Washington within the Glacier Peak Wilderness Area of the Wenatchee National Forest. Its active front now terminates in a lake at ca. 1,900 m a.s.l. elevation, having receded about a kilometer from its terminal moraine of 1895 at an elevation of ca .1,800 m a.s.l. (Fig. 1). The time any given spot on the glacier forefront was exposed from under the receding glacier can be estimated within a few years from photographs taken since the late nineteenth century, including aerial photos since the early 1950s, supplemented with counts of annual branch whorls on trees. It receded 533 m from 1895 to 1940, an average of about 12 m per year (Freeman 1941). Between 1940 and 2001 it additionally receded an average of ±8 m per year for an 1895–2001 total of ±1 km. Recession in length has slowed in recent years, but the glacier has been losing depth and its terminus in the lake has become concave rather than convex as it was a few decades ago.

A well-defined terminal moraine, scattered inconspicuous recessional moraines, fluting, and outwash characterize the glacier forefront. Precipitation at a nearby snow survey station averages 2,750 mm per year, mostly as snow, and the growing season is about 3 months between disappearance of snow pack in early- to mid-July to onset of fall frost and snow in early October. Having experienced negligible anthropogenic disturbance and lacking introduced plants, the forefront presents an excellent outdoor laboratory to test the hypothesis stated above. Its relatively pristine condition is due largely in part to difficulty of access, which entails lake travel by boat for some 25 and 16 km of trail into the Wilderness Area. The forefront's change in elevation of only about 100 m over a kilometer minimizes the effects of elevation on its ecology.

Adjacent to the forefront are secondary-successional dry meadow communities on the lower slopes of a low ridge that we designated "Glacier View". Nearby, Cloudy Peak has high alpine communities at the upper limits of vascular plant growth at ca. 2,600 m a.s.l. elevation. These communities provided a comparison to the primary successional communities on the forefront.

Succession of ectomycorrhizal fungi in the primary successional plant communities of the forefront and adjacent secondary successional communities of Glacier View has been reported by Jumpponen et al. (1999a,b, 2002). Soil carbon and nitrogen contents along the chronosequence were reported by Ohtonen et al. (1999).

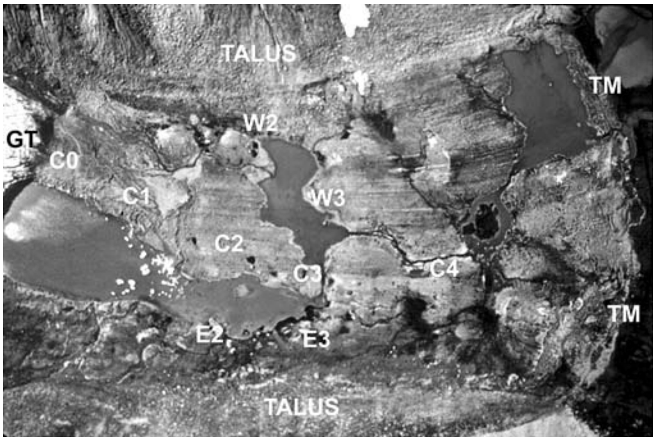

Fig. 1 Aerial photo of Lyman Glacier forefront; distance from the present glacier terminus (*GT*) to the terminal moraine of 1895 (*TM*) is ca. 1 km; the east and west edges of the forefront are bounded by talus at the base of cliffs. Sampling points on the chronosequence transects are *W2* and *W3* on the west boundary; *C0–C4* down the forefront center, and *E2* and *E3* on the east boundary. The striations from left to right on the forefront are glacial flutings

Chronosequence sampling plan

Colonizer communities on glacial forefronts present sampling problems because of wide spacing of plants and nonrandom distributions. Slumping, frost heaving, stream washing, and snow deposition can locally prevent plant establishment (Matthews 1979a). All plant taxa encountered over the forefront's most recent 65 years of exposure from under the retreating glacier were sampled at sites along three transects leading from the present glacier terminus to the terminal moraine to determine the chronosequential fungal colonization of roots on the forefront: (1) along the east boundary of the forefront (E), (2) down the center (C), and (3) along the west boundary (W).

Transect E included a series of small lateral moraines and ran along the lake in which the active flow of the glacier now terminates (Fig. 1). Cliffs, including vegetated benches, rise from talus above the east transect, which is paralleled by an informal trail to Spider Gap used by wildlife and hikers.

Transect C started at the dormant edge of the glacier terminus on a peninsula between the terminus lake to the East and mud flats to the West (Fig. 1). Because the peninsula dead-ends at the glacier, only mountain goats occasionally use it as a through route. However, bear, deer and elk tracks were observed on the peninsula.

Transect W ran from the glacier terminus between the mud flats noted above and talus, cliffs and benches of the main north-south ridge extending north from Chiwawa Mountain, the originating peak of Lyman Glacier (Fig. 1). This ridge, the crest of the North Cascades, was the source of avalanche debris of soil, roots and broken tree remnants scattered along the west transect. No signs of animal traffic were noted along this transect.

On the central transect, site 0 was within 4 m of the glacier terminus and had been exposed for about 15 years; site 1 was exposed about 25 years, site 2 about 35 years, site 3 about 45 years and site 4 about 60 years. On the east and west transects only sites 2 and 3, representing equivalent exposure times as the sites with the same numbers in the central transect, were sampled.

Plant species composition and dominance

At each site, 10–15 1-m^2 quadrants clustered in a 4×4 grid were laid out as described by Matthews (1979a,b) for studies of forefronts. To determine relative dominance, all species were identified and counted within each 1-m^2 quadrant in which any aerial part of a species occurred (Matthews 1979a). This single measure combines number, size, shape and pattern, is easily measured for species of diverse life-form, is sensitive where cover and density are low, and varies relatively little as the phenological aspect of the vegetation fluctuates through the short growing season (Matthews 1979a).

Plant species and cover dominance were determined for all sample sites except W3, which experienced late snow cover and frequent disturbance by rocks falling from cliffs, the Glacier View dry meadow, which had a nearly complete plant cover; or the Cloudy Peak high alpine site, where plants were too widely spaced for application of this method.

Mycorrhizal propagule content of soils

A bioassay for the presence of viable fungal propagules was conducted with soil sampled from open areas between plants at sites C0, C2, E2, and W2, as well as in the fully vegetated Glacier View dry meadow. Seeds of *Anemone occidentalis*, *Aster alpigenus*, *Epilobium latifolium*, *Petasites frigidus* and *Vaccinium deliciosum* were germinated and transplanted to 200 cm^3 Leach tubes (Leach, Corvallis, Ore.) with a mix 1:1 soil and "leca" (expanded clay pellets). Members of the Pinaceae were not included: Jumpponen et al. (2002) have already reported results of most probable number tests for ectomycorrhizal inoculum in forefront substrate, and soil DNA analysis by Jumpponen (2003) showed the presence of EM and DS propagules even in nonvegetated substrates close to the glacier terminus. Control treatments consisted of the same mixture for each soil sample autoclaved. No fertilizer was applied to the soil. All treatments were replicated five times.

Root sampling

For our purpose, a sampling of the mycorrhizal status of just a portion of the root system and surrounding soil of a large number of plants was deemed more informative than a detailed assessment of entire root systems of fewer plants. This approach also minimized disturbance to the fragile communities, as required by our collecting permit from the United States Forest Service, and reduced the logistical difficulties in the 2 days required for transporting samples by trail, boat and automobile to the laboratory for analysis. Entire root systems were collected only from very small plants.

The general goal of replication was to sample three plants of each species at each site. More were collected of species that were abundant or dominant at individual sites. In several cases a species was so infrequent at a site that we sampled only one or two to avoid extirpating it at that site.

Determination of mycorrhizal colonization

Root samples were cut into segments and cleared and stained to determine mycorrhizal colonization by a modification of the method of Phillips and Hayman (1970). Roots were cleared in 10% KOH solution, steamed for 30 min, rinsed with tap water, transferred to 1% HCl solution for 30 min, then rinsed again with tap water. Cleared samples were transferred into a staining solution of 0.5% Trypan-Blue in lactoglycerol, steamed for 30 min, rinsed with tap water and stored in cold water or lactoglycerol solution until microscopic examination. Colonization was confirmed by use of a compound microscope but quantified for AM and DS as proportion of root length colonized by scanning with a stereomicroscope in a modification of the method described by Kormanik and McGraw (1982), or for EM and ERM as proportion of feeder rootlets colonized. Proportions colonized were broadly categorized as percentage of fine root length with AM or DS or of feeder rootlet numbers with EM or ERM: NM = 0; + = 1–25%; ++ = 26–50%; +++ = 51–75%; ++++ = 76–100%. These broad categories were used because only portions of most plant root systems were examined; variation of colonized roots within subsamples of single plant root systems was so high that greater precision of measurement would not yield more precise interpretations.

DS colonization was characterized by melanized, thick-walled, septate hyphae, usually forming microsclerotia within the root cells, or sometimes simply colonizing the root cells with meandering hyphae. AM were considered present if vesicles, arbuscules and/or strongly staining, broad, aseptate hyphae were present in root cells. Vesicles and aseptate hyphae were more common than arbuscules. ERM were distinguished as brown, thin-walled, septate hyphae forming coils or clumps of hyphae inside outer root cells of the Ericaceae. EM was judged by the presence of a Hartig net and/or mantle.

Results

Plant species composition and dominance

In all, 504 plants representing 22 families, 44 genera and 66 species were evaluated for mycorrhizal colonization. Communities are described below by the three chronosequence transects and the two dry meadow communities near the forefront.

Central chronosequence

Site C0, closest to the base of the glacier, has been free of ice for ca. 15 years and lacked vegetation.

Site C1 (Table 1), exposed ca. 25 years, had a widely spaced plant community of 11 species, with cover co-dominants being *Juncus mertensianus*, *Juncus drummondii*, and *Saxifraga ferruginea*. Three conifer species were also present as new seedlings in the 1st year of sampling: several *Abies lasiocarpa* and one each *Picea engelmannii*, and *Pinus contorta*. They persisted through the 3rd year of the study, growing about 1 cm in height in both the second and third growing seasons. During that time several other *Abies lasiocarpa* established. The *P. contorta* seedling was the only representative of that species seen in the Lyman Glacier basin.

Site C2 (Table 1), exposed ca. 35 years, had 28 species with *Salix phylicifolia* and *Juncus drummondii* as cover co-dominants. Other common species were *Minuartia rubella*, *Luzula piperi*, *Saxifraga ferruginea*, *Epilobium latifolium*, *Phyllodoce empetriformis*, and *Vaccinium deliciosum*. The *Phyllodoce* and *Vaccinium* sometimes established under the low crowns of the *Salix* to form patches of vegetation. Scattered gymnosperms, especially *Abies lasiocarpa* and *Tsuga mertensiana*, occurred mostly in the protection of large stones but occasionally on raised ground.

Site C3 (Table 1), exposed ca. 45 years, had 15 species with *Epilobium latifolium*, *Salix phylicifolia* and *Phyllodoce empetriformis* as cover co-dominants. Other common species included *Saxifraga ferruginea*, *Luzula piperi*, *Juncus drummondii*, *Luetkea pectinata* and *Carex nigricans*. Plant spacing was still open, with much bare ground between plants of plant patches. Scattered gymnosperms were present as in C2.

Site C4 (Table 1), exposed ca. 60 years, had 18 species with *Salix phylicifolia*, *Phyllodoce empetriformis* and *Juncus drummondii* as cover co-dominants. Other common species were *Epilobium latifolium*, *Saxifraga ferruginea*, *Luzula hitchcokii*, *Cassiope mertensiana* and *Tsuga mertensiana*. This site still had much open ground on which few or no plants had established.

East chronosequence

Site E2 (Table 1), exposed ca. 35 years, had 26 species, with *Phyllodoce empetriformis* and *Cassiope mertensiana* as cover co-dominant species. Other common species included *Juncus drummondii*, *J. mertensianus*, *V. deliciosum*, *Luetkea pectinata* and *Tsuga mertensiana*. In contrast to site C2, the *Phyllodoce* and *Cassiope* at E2 established primarily in open-to-protected niches rather than under *Salix* crowns.

Site E3 (Table 1), exposed ca. 45 years, was perennially wet from snowmelt. Of its 21 species, *Luetkea pectinata*, *Juncus drummondii* and *Phyllodoce empetriformis* were cover co-dominants. Other common species included *Vaccinium deliciosum*, *Luzula piperi* and *Poa cusickii.* The site bordered on a wet area that contained wetland-adapted species such as *Carex spectabilis* and *Juncus mertensianus*.

West chronosequence

Site W2 (Table 1), exposed ca. 35 years, had 21 species with *Juncus drummondii* and *Mimulus tilingii* as cover co-dominants. Other common species were *Juncus mertensianus*, *Epilobium alpinum*, *Minuartia rubella*, and *Saxifraga tolmiei*. This site was perennially wet from runoff from cliffs above and from melting of snow that persisted well into summer, a habitat particularly suited to *Mimulus tilingii.*

Site W3 (Table 1), exposed ca. 45 years, was characterized by a scattering of eight species, none being dominant. In the shade of cliffs and subject to snow and rock avalanches, it was perennially wet and under snow cover into midsummer.

Table 1 Mycorrhizal status of plants from the Lyman Glacier forefront chronosequence sites at different years since exposure from under the retreating glacier, and from the Glacier View (*GV*) dry meadow and the high alpine site on Cloudy Peak (*CP*). Forefront transect sites: *C* Central, *E* east, *W* west; mycorrhizal status: *NM* nonmycorrhizal, *AM* arbuscular mycorrhizal, *EM* ectomycorrhizal, *ERM* ericoid mycorrhizal, *DS* dark septate endophyte; proportion of roots colonized (intensity): + 1–25%, ++ 26–50%, +++ 51–75%, ++++ 76–100%

Plant taxon	Site	Age (years)	Colonization type with range of intensity and number of plants observed				
			NM	AM	EM	ERM	DS
Angiosperms-monocotyledons							
Cyperaceae							
Carex nigricans C.A. Mey	C1	25	3				
	W2	35	1	1:+			1:+
	E3	45	1				
Carex scopulorum T. Holm	C2	35	2				1:++
	E2	35	2				1:+
	W2	35	1				
	C3	45	2				1:+
Carex spectabilis Dewey	E3	45	2				
Carex sp	C4	60	4				
	GV	Old		1:+			1:+++
Juncaceae							
Juncus drummondii Steud	C1	25	3				
	C2	35	4				2:++/+++
	E2	35	6				1:++
	W2	35	2	1:+++			2:+
	C3	45	2				1:+
	E3	45	3				
Juncus mertensianus Bong	C1	25	3				
	C2	35	3				
	W2	35	2	1:+			1:+
	C3	45	2				1:++
	E3	45	2				1:+
	W3	45	2				1:+
Luzula piperi (Coville) M.E. Jones	C1	25	2				1:+
	C2	35	2				1:+
	E2	35	2	1:+			2:+
	W2	35	1	1:+			2:+
	C3	45	1				2:+
	E3	45	2				
	C4	60					3:+/++
	GV	Old		1:+			1:+++
Liliaceae							
Erythronium grandiflorum Pursh	GV	Old		1:++			1:++
Veratrum viride Ait	GV	Old		1:++			1:++
Melanthiaceae							
Tofieldia glutinosa Pers	E3	35					1:+
Poaceae							
Agrostis variabilis Rydb	E3	45	2				
Deschampsia cespitosa (L.) P. Beauv	E2	35	1	2:+/++			1:+
Festuca sp	GV	Old					1:++
Poa cusickii Vasey	E2	35	2	1:++			1:+
Poa nevadensis Vasey	C2	35	1	4:+/+++			4:+/++
Poa sp	W2	35	1	2:+/+++			3:+/++
Grass sp	GV	Old		2:+			2:+/++
Angiosperms—dicotyledons							
Asteraceae							
Anaphalis margaritacea Benth. & Hook	GV	Old		1:+			1:++
Aster alpigenus (Torr. & A. Gray) A. Gray	C2	35		4:+/++			4:++/++++

Table 1 (continued)

Plant taxon	Site	Age (years)	Colonization type with range of intensity and number of plants observed				
			NM	AM	EM	ERM	DS
Crepis nana Richardson	W2	35		3:+/++			
	E3	45		2:+++			1:+
Erigeron aureus Greene	CP	Old		1:++			1:++
Senecio cymbalarioides H. Buck	E3	45		1:+++			1:++
Senecio fremontii Torr. & A. Gray	C2	35	3				
	W2	35	1		3:++/+++		
Brassicaceae							
Draba oligosperma Speg	CP	Old					1:+
Caryophyllaceae							
Minuartia rubella Hiern	C1	25	3				
	C2	35	2				1:+
	W2	35	3				
	C3	45	2				
	W3	45	3				
	C4	60	4	1:++			2:+/++
Ericaceae							
Cassiope mertensiana G. Don	C2	35				3:++/++++	
	E2	35				2:++	
	W2	35				1:+	
	C3	45				4:+/++++	1:++
	E3	45				3:+/++	1:+
	C4	60				3:+/++++	2:++/++++
	GV	Old				1:+	
Ledum glandulosum Nutt	E3	45				3:+/+++	1:++
	C4	60				2:+/+++	2:+/++
Phyllodoce empetriformis D. Don	C2	35				3:+/+++	1:++++
	E2	35				7:++/++++	3:+
	W2	35				4:+/+++	3:+
	C3	45				3:++/++++	3:+/+++
	E3	45				2:+++	
	C4	60				3:+/++++	3+/+++
	GV	Old				1:++++	1:++
	CP	Old	1	1:+++		1:+	1:+++
Phyllodoce glanduliflora (Hook.) Coville	E2	35				7:++/+++	5:+/+++
	W2	35				2:+/+++	2:+/++
	E3	45				3:+++/++++	1:++
Vaccinium caespitosum Michx	CP	Old					1:+
Vaccinium deliciosum Piper	C2	35				3:+/++++	
	E2	35				4:++/++++	4+/++++
	W2	35					2:+/++
	C3	45				3:++/+++	1:++
	E3	45				3:++/+++	2:+
	C4	60				5:+/++++	3:++
	GV	Old				1:+++	1:++
Fabaceae							
Lupinus lepidus Dougl	GV	Old					1:++
Onagraceae							
Epilobium alpinum L	C1	25	3				
	C2	35	1	5:+/++++			4:+/+++
	E2	35		1:+++			
	W2	35	2	1:+			2:+
	W3	45	3				
Epilobium angustifolium L	GV	Old		1:+			1:++

Table 1 (continued)

Plant taxon	Site	Age (years)	Colonization type with range of intensity and number of plants observed				
			NM	AM	EM	ERM	DS
Epilobium latifolium L	C2	35	4				1:+
	E2	35		1:+			1:+
	W2	35		3:+/+++			2:++
	W3	45	3				
	C4	60	1	2:+/++			
Epilobium watsonii Barbey	E2	35	1				
	E3	45	1				
Polemoniaceae							
Phlox diffusa Benth	CP	Old		1:++			1:+
Polygonaceae							
Oxyria digyna Hill	C1	25	2				
	C2	35	3				
	W2	35	2	3:+/++			5:+/+++
	W3	35	1				
	C4	45	1				
Polygonum bistortoides Pursh	C2	60			1:+		
	GV	Old			1:+		1:+++
Ranunculaceae							
Anemone occidentalis S. Watson	GV	Old		1:+++			1:++
	CP	Old		1:+			1:+
Rosaceae							
Luetkea pectinata Kuntze	C2	35	1	3:+			2:+/++
	E2	35		3:+/++++			1:+++
	C3	45		2:+			2:+
	C4	60		3:+/+++			2:++/+++
	GV	Old		3:+			2:++
	CP	Old					1:+
Potentilla flabellifolia Hook	GV	Old		1:++++	1:+		1:++++
Sorbus sitchensis M. Roem	GV	Old		1:+++	1:+		1:+
Salicaceae							
Salix commutata Bebb	C2	35			5:++/++++		4:+/++++
	E3	45		2:+/++	2:+++		2:+/++++
	C4	60		1:++	4:+/++++		3:+++/++++
Salix nivalis Hook	W2	35		1;++	2:++/+++		2:+
Salix phylicifolia L	C1	24			1:+++		1:+
	C2	35		1:++	3:+/+++		3:++/++++
	W2	35			2:++/+++		2:+
	C3	45			4:++/++++		5:+/++++
	E3	45			2:+++/++++		1:+
	C4	60			4:+/+++		3:++/+++
Saxifragaceae							
Saxifraga ferruginea Graham	C1	25	2				1:+
	C2	35	2				1:+
	E2	35	3	1:+			1:+
	C3	45	5				
	E3	45	3				1:+
	C4	60	7				1:+
Saxifraga punctata L	E2	35		1:+			1:+

Table 1 (continued)

Plant taxon	Site	Age (years)	Colonization type with range of intensity and number of plants observed				
			NM	AM	EM	ERM	DS
Saxifraga tolmiei Torr. & A. Gray	C1	25	4				
	C2	35	8				1:+
	E2	35	3				
	C3	45	2				
	W3	45	1				1:+
	C4	60	6				
Scrophulariaceae							
Castilleja parviflora Bong	C2	35	1	3:+			
Mimulus lewisii Pursh	C1	25	3	1:+			
	C2	35		2:+			
	E2	35		1:+++			
	W2	35		3:++/+++			1:+
Mimulus tilingii Regel	C2	35	1				
	E2	35	2	1:+			1:+
	W2	35	1	3:+/++			
	C3	45	3				
	W3	45					1:+
	C4	60	2				
Pedicularis attollens A. Gray	E2	35	3				
	E3	45	3				
Pedicularis bracteosa Benth	GV	Old					1:++++
Pedicularis groenlandica Retz	C2	35	2				1:+++
	E2	35		1:++			1:+
	C3	45	1				2:++
	E3	45	1				
Pedicularis sp	GV	Old					1:+
Penstemon davidsonii Greene	C2	35	1	1:++			
	W3	45					1:+
Veronica cusickii A. Gray	W2	35		4:++/+++			2:+/++
	GV	Old					1:+++
Veronica wormskjoldii Roem. & Schult	C2	35		3:+			3:+/+++
	E2	35		6:+/++++			
	W2	35		3:+/+++			1:+
	C4	60		4:+			2:+/+++
Gymnosperms							
Pinaceae							
Abies lasiocarpa (Hook.) Nutt	E2	35			3:++/+++		1:+
	C3	45			2:+/++++		2:+
	C4	60			5:++/+++		1:+
	E2						
Larix lyalii Parl	E2	35			2:+/++++		
Picea engelmannii Parry	E2	35			2:++		
Tsuga mertensiana Carriére	C2	25			3:+++		3:+/++
	E2	25			5:++/++++		3:+/+++
	E3	25			2:++/+++		2:+
	C4	60			2:++/+++		2:+/++
Monilophytes							
Equisetaceae							
Equisetum arvense L	E2	35		1:+			
Pteridophytes							
Athyriaceae							
Cystopteris fragilis (L.) Bernh	C2	35		3:+/+++			
Cryptogrammaceae							
Cryptogramma crispa (L.) R. Br	W2	35		4::+/++			

Glacier view dry meadow

Rising gently from the 1895 terminal moraine of Lyman Glacier, this area includes heathlands, wet and dry meadows, and conifer parkland. The soil has not been glaciated at least for nearly 7,000 years, judging from its undisturbed layer of Mount Mazama ash (W. Long, personal communication). However, photos taken in ca. 1895 show effects of recent fire. The 21 species, all perennials in the Poaceae, Ericaceae, and other, less frequent families, provided nearly 100% cover (Table 1). These, along with nearby conifers, would be a potential seed source for species establishing on the forefront.

Seven widely spaced species were sampled at the high alpine site on Cloudy Peak. None were dominant, although *Luetkea pectinata*, *Phlox diffusa* and *Phyllodoce empetriformis* formed sizeable mats.

Soil propagule content

Bioassay plants grown on samples from the forefront produced no AM colonization, although spores of *Glomus fasciculatum* (Thaxter) Gerd. and Trappe sensu stricto were present among roots of occasional sampled plants in the forefront itself. Soil from the Glacier View dry meadow produced high levels of AM on all bioassay plant species except *Vaccinium deliciosum*.

Four containers with *Vaccinium deliciosum* formed ERM in the autoclaved controls suggesting aerial contamination in the growth room, so no conclusions can be drawn regarding ERM propagules. DS colonization was observed in at least one seedling of all five plant species in soils from all forefront and Glacier View sites.

Mycotrophy of the plant communities

In the central chronosequence, the proportion of plants with colonized roots increased with increased time of exposure of the site from under the ice. The reverse was true for the East and West transects, where avalanche deposits and high soil water content from late snowmelt and seepage from upslope affected the sites differentially. Proportions of the plants that were NM, AM, ERM, EM or DS are shown for all sampling sites on the three chronosequences in Fig. 3. AM were generally low (<30% of the plants) in all but site W2 (49%), and nearly or altogether absent in sites C1 and W3. ERM were present in all Ericaceae, and EM in all Pinaceae and Salicaceae sampled. DS occurred at moderate rates (24–46% of the plants) in all chronosequence sites except C1, in which only 13% of the sample plants were colonized. DS occurred in all plants in the long established communities of Glacier View.

The mycorrhizal status of all species sampled is shown in Table 1 by site, age of substrate, and category of colonization. An impression of the comparative intensity of colonization by mycorrhiza type of an individual species can be visualized for different ages of substrate by scanning the symbols for colonization intensity.

Central chronosequence

Site C1 (Table 1, Fig. 2): 26 of the 31 sampled plants (84%) were NM, 4 (13%) had low levels of DS, 1 *Mimulus lewisii* had a low level of AM, and 1 *Salix phylicifolia* had a high level of EM along with some DS. The few seedlings of *Abies lasiocarpa*, *Picea engelmannii*, and *Pinus contorta* at C1 were not sampled; to have done so would have obliterated them from this earliest community.

Site C2 (Table 1, Fig. 2): 45 of the 105 sampled plants (43%) were NM, 37 (35%) had low to high levels of DS, 29 (28%) had low-to-high levels of AM, 12 (11%) had EM,

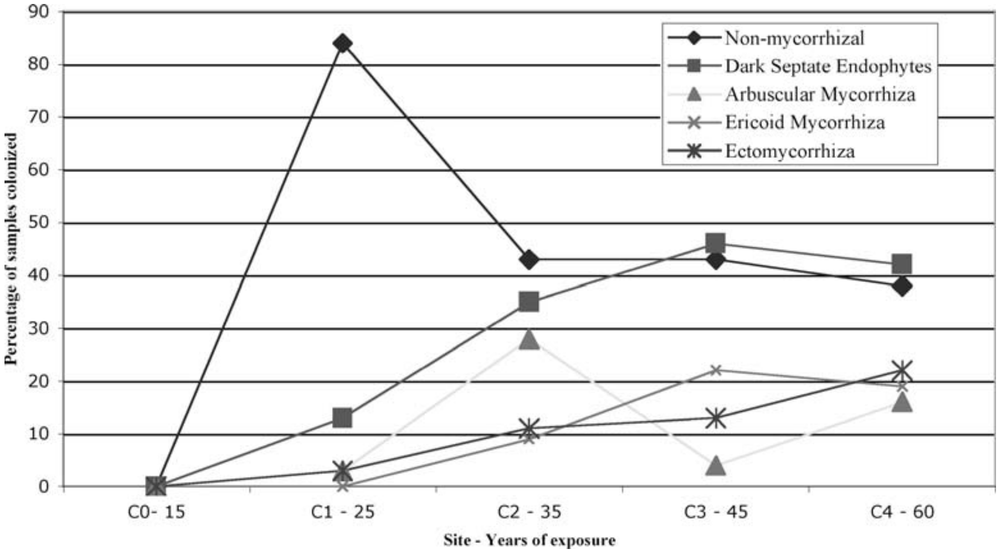

Fig. 2 Frequency of mycorrhizal colonization (in percent) of all plants sampled along the central chronosequence in the Lyman Glacier forefront

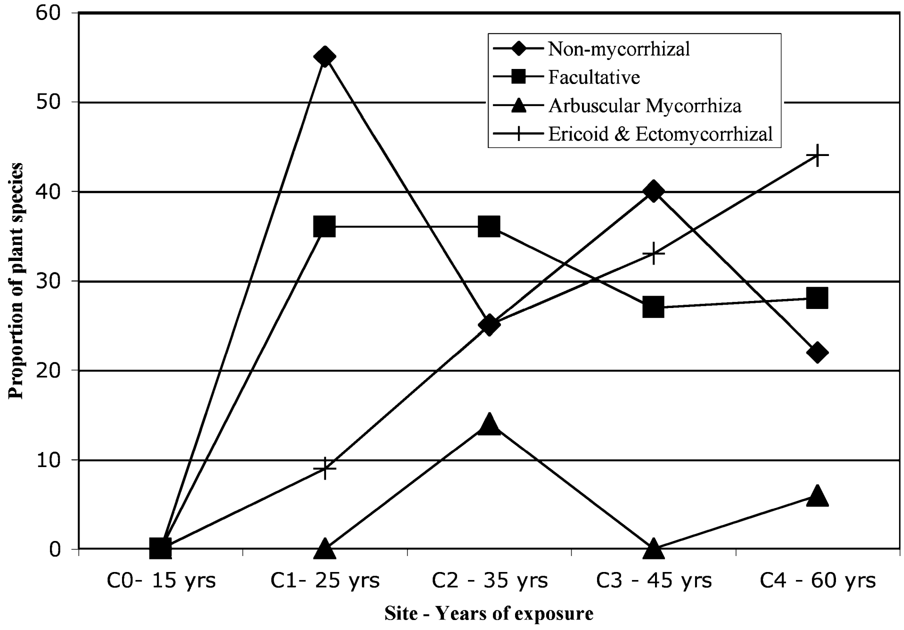

Fig. 3 Proportion of plant species of different mycotrophic habit along the central chronosequence in the Lyman Glacier forefront

and 9 (9%) had ERM. One *Salix phylicifolia* specimen had DS, EM and AM.

Site C3 (Table 1, Fig. 2): 20 of the 46 plants sampled (43%) were NM, 21 (46%) had low-to-high levels of DS, 2 (4%) had low levels of AM, 10 (22%) had low-to-high levels of ERM and 6 (13%) had low-to high levels of EM.

Site C4 (Table 1, Fig. 2): 26 of the 69 plants sampled (38%) were NM, 29 (42%) had low-to high levels of DS, 11 (16%) had low-to-high levels of AM, 13 (19%) had low-to-high levels of ERM and 15 (22%) had low-to-high levels of EM. One *Salix phylicifolia* plant had DS, AM and ERM.

East chronosequence

Site E2 (Table 1): 25 of the 85 plants sampled (29%) were NM, 30 (35%) had low-to-high levels of DS, 23 (27%) had low-to-high levels of AM, 20 (23%) had low-to-high levels of ERM and 12 (14%) had EM.

Site E3 (Table 1): 21 of the 47 plants sampled (45%) were nonmycorrhizal, 15 (32%) had low-to-high levels of DS, 6 (13%) had low-to-high levels of AM, 5 (11%) had hyphae and vesicles that resembled AM, 14 (30%) had low-to-high levels of ERM and 6 (13%) had high levels of EM. One *Salix commutata* had DS, AM and EM.

West chronosequence

Site W2 (Table 1): 17 of the 76 plants sampled (22%) were NM, 31 (41%) had low-to-high levels of DS, 37 (49%) had low-to-high levels of AM, 10 (13%) had low-to-high levels of ERM and 4 (5%) had low-to-high levels of EM. One *Salix nivalis* had DS, AM and EM.

Site W3 (Table 1): 13 of the 17 plants sampled (76%) were NM and 4 (24%) had low levels of DS. AM, ERM and EM were absent. Facultative mycorrhizal hosts, which can form mycorrhizae but can survive without them in natural soils such as *Epilobium alpinum*, *E. latifolium*, *O. digyna* and *Penstemon davidsonii*, were all NM here. ERM and EM hosts were absent.

Glacier View dry meadow (Table 1): all plants had DS, all Ericaceae also had ERM, and the majority of the others had AM. No willows or conifers were present in the quadrats, but nearby medium-to-large *Abies lasiocarpa* and *Tsuga mertensiana* were strongly EM.

Cloudy Peak high alpine (Table 1): all plants had DS, 4 of the 8 also had AM, and one ericaceous species had ERM. The site was far above the upper limit of willows or conifers.

Mycotrophy of plant species by family and genus

In general, the plant families recorded at Lyman Glacier as being NM, or having AM, ERM, EM or DS (Tables 1, 2), conformed to what would be predicted from the lists of Read and Haselwandter (1981), Harley and Harley (1987), Newman and Reddell (1987), Trappe (1988), Jumpponen and Trappe (1998), and others. Some exceptions to general trends and some novelties deserve mention.

NM samples occurred in all families except the Equisetaceae, which was represented by only one sample so no general inferences could be drawn. The high incidence of NM individuals in the Saxifragaceae was notable but due mostly to the large number of individuals of *S. ferruginea* sampled: 22 of 27 plants of this species were NM. Samples from 4 of the 27 individuals of the Pinaceae were NM, but these were small samples from rather large individual root systems.

DS endophytes were by far the most widely distributed root colonizers. Occasional individuals of predominantly NM genera had AM, i.e., were facultative mycorrhiza

Table 2 Plant families from the Lyman Glacier forefront and their mycorrhizal status (families represented by only one individual are omitted)

Family	Number of samples	Mycorrhizal status				
		NM	AM	EM	ERM	DS
		Samples colonized (%)				
Monocotyledons						
Cyperaceae	22	82	4	0	0	18
Asteraceae	17	23	76	0	0	35
Juncaceae	67	64	6	0	0	34
Poaceae	20	35	45	0	0	45
Dicotyledons						
Asteraceae	17	24	76	0	0	35
Caryophyllaceae	21	81	5	0	0	14
Ericaceae	78	2	1	0	97	51
Onagraceae	34	56	38	0	0	38
Polygonaceae	16	56	19	7	0	31
Rosaceae	13	8	92	0	0	54
Salicaceae	31	3	16	94	0	84
Saxifragaceae	54	85	4	0	0	13
Scrophulariaceae	67	36	54	0	0	24
Gymnosperms						
Pinaceae	27	4	4	96	0	52
Pteridophytes						
Athyriaceae	3	0	0	0	0	100
Cryptogrammaceae	4	0	0	0	0	100

formers: *Minuartia* (Caryophyllaceae), *Carex* (Cyperaceae), *Juncus* and *Luzula* (Juncaceae), and *Oxyria* (Polygonaceae). One *Oxyria* digyna plant more than 1 m distant from other plants, and sampled in its entirety, had a totally NM root system but a large cluster of *Glomus fasciculatum* spores had formed among its roots.

Polygonum bistortoides (Polygonaceae) is common in Glacier View communities but rare on the glacier forefront. The single samples from each of those sites had a low level of EM (Table 1). Lesica and Antibus (1986) reported AM on this species.

Discussion

These studies were conducted in an area with little anthropogenic disturbance. The results support the hypothesis that plant colonization is related to the availability of propagules of mycorrhizal fungi (Trappe 1988; Trappe and Luoma 1992). When first deglaciated, the substrate is a parent material subject to settling, slumping, and frost heaving, and with low microbial activity and few or no propagules of mycorrhizal fungi. After 15–20 years, this substrate stabilizes enough that plants can establish in “safe sites” that trap seeds and protect seedlings from the harsh environment. Host plants that can survive without mycorrhizae prevail in this earliest stage of primary succession. However, some DS, EM, and ERM plants also occur in the earliest stage, confirming the early availability of the fungal propagules shown by soil DNA analysis (Jumpponen 2003), probably by aerial dispersal.

As plants in the earliest colonized sites age, organic matter slowly accumulates in the soil, roots of perennials begin to pervade the bare ground between plant crowns, forming mycorrhizae there as a potential inoculum for new seedlings. DS, ERM and EM plant species become dominant over time. New seedlings of various species continue to establish in “safe” sites and may establish near or under the crowns of *Salix* shrubs. As the soil under the *Salix* accumulates organic matter, microbial and mycorrhizal fungus populations increase.

Meanwhile, the EM fungal diversity and soil microbial biomass and functions increase. Forcibly discharged EM spores of epigeous fungi are dispersed to the forefront by wind, whereas the statismospores of hypogeous found on the forefront would be introduced in feces of visiting mycophagous animals (Cázares and Trappe 1990, 1994). AM spores arrive mostly in stochastic events: those borne singly in soil can be introduced with movement of that soil in avalanches from cliffs that surround the forefront, or by falling from the feet of visiting ungulates (in this mesic subalpine habitat, soil that contains AM spores is generally damp and held in place by the host roots so it would be wind-borne to the forefront only exceptionally). Alternatively, AM spores may be delivered in the feces of mycophagous animals, although we did not find any in fecal deposits on the forefront.

Our data demonstrate a relationship between mycorrhiza types and plant establishment and succession, but not cause and effect. However, volumes of past research have shown that terrestrial, woody perennials in the northern hemisphere are strongly mycorrhiza dependent, the more so in infertile soils. New and startling evidence would be needed to effectively argue that the Pinaceae and *Salix* spp. that dominate the forefront could have done so without mycorrhiza formation. Until such evidence is produced, the availability of mycorrhizal fungi can be said to set the patterns of plant succession on the Lyman Glacier forefront in conjunction with other environmental phenomena.

Our results also reinforce the observations of Peyronel (1924), Read and Haselwandter (1981), Currah and Van Dyk (1986), Blaschke (1991), and Jumpponen (1999) that DS fungi abound in subalpine and alpine systems. The biology and role of these endophytes clearly deserves high priority in research programs designed to clarify the functions and interactions of these important organisms in belowground ecosystems.

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References

Allen EB, Allen MF (1984) Competition between plants of different successional stages: mycorrhizae as regulators. Can J Bot 62: 2625–2629

Allen EB, Chambers JC, Connor KF, Allen MF, Brown RW (1987) Natural reestablishment of mycorrhizae in disturbed alpine ecosystems. Arctic Alp Res 19:11–20

Allen MF (1991) The ecology of mycorrhizae. Cambridge University Press, Cambridge, UK

Blaschke H (1991) Multiple mycorrhizal associations of individual calcicole host plants in the alpine grass-heath zone. Mycorrhiza 1:31–34

Cázares E, Trappe JM (1990) Alpine and subalpine fungi of the Cascade Mountains. I. *Hymenogaster glacialis* sp. nov. Mycotaxon 38:245–249

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

Currah RS, Van Dyk M (1986) A survey of some perennial vascular plant species native to Alberta for occurrence of mycorrhizal fungi. Can Field Nat 100:330–342

Dominik T (1961) Studium o mikorhyzie. Folia For Pol Ser A 5:3–160

Freeman O (1941) The recession of Lyman Glacier, Washington. J Geol 49:764–771

Harley JL, Harley EL (1987) A check-list of mycorrhiza in the British flora. New Phytol 105[Suppl]:1–102

Hayman DS (1982) Influence of soils and fertility on activity and survival of vesicular-arbuscular mycorrhizal fungi. Phytophathology 72:1119–1125

Helm DJ, Allen EB, Trappe JM (1996) Mycorrhizal chronosequence near Exit Glacier, Alaska. Can J Bot 74:1496–1996

Jumpponen A (1999) Spatial distribution of discrete RAPD phenotypes of a root endophytic fungus, *Phialocephala fortinii*, at a primary successional site on a glacier forefront. New Phytol 141:333–344

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

Jumpponen A, Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol 140:295–310

Jumpponen A, Mattson K, Trappe JM (1998a) Mycorrhizal functioning of *Phialocephala fortinii* with *Pinus contorta* on glacier forefront soil: interactions with soil nitrogen and organic matter. Mycorrhiza 7:261–265

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

Jumpponen A, Váre H, Mattson KG, Ohtonen R, Trappe JM (1999b) Characterization of 'safe sites' for pioneers in primary succession on recently deglaciated terrain. J Ecol 87:98–105

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

Kormanik PP, McGraw AC (1982) Quantification of arbuscular mycorrhizae in plant roots. In: Schenk NC (ed) Methods and principles of mycorrhizae research. American Phytopathological Society, St. Paul, Minn., pp 37–46

Kohn LM, Stasovski E (1990) The mycorrhizal status of plants at Alexandra Fiord, Ellesmere Island, Canada, a high arctic site. Mycologia 82:23–35

Lesica P, Antibus RK (1986) Mycorrhizae of alpine fell-field communities on soils derived from crystalline and calcareous parent materials. Can J Bot 64:1691–1697

Matthews JA (1979a) The vegetation of the Storbreen gletschervorfeld, Jotunheimen, Norway. I. Introduction and approaches involving classification. J Biogeogr 6:17–47

Matthews JA (1979b) The vegetation of the Storbreen gletschervorfeld, Jotunheim, Norway. II. Approaches involving ordination and general conclusions. J Biogeogr 6:133–167

Newman EI (1988) Mycorrhizal links between plants: their functioning and ecological significance. Adv Ecol Res 18:241–270

Newman EI, Reddell P (1987) The distribution of mycorrhizas among families of vascular plants. New Phytol 106:745–751

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

Peyronel B (1924) Prime ricerche sulla micorize endotrofiche e sulla microflora radicola normale della fanerogame. Riv Biol 6:17–53

Phillips JM, Hayman DS (1970) Improved procedure for clearing roots and staining parasitic and arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Read DJ, Francis R, Finlay RD (1985) Mycorrhizal mycelial and nutrient cycling in plant communities. In: Fitter AH, Atkinson D, Read DJ, Usher MB (eds) Ecological interactions in soil. Blackwell, Oxford, pp 193–218

Read DJ, Haselwandter K (1981) Observations on the mycorrhizal status of some alpine plant communities. New Phytol 88:341–352

Reeves FB, Wagner D, Moorman T, Kiel J (1979) The role of endomycorrhizae in revegetation practices in the semi-arid West. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. Am J Bot 66:6–13

Sengupta A, Chakraborty DC, Chaudhuri S (1989) Do septate endophytes also have a mycorrhizal function for plants under stress? In: Mahadevan A, Raman N, Natarajan K (eds) Mycorrhizae of green Asia. Proceedings of First Asian Conference on Mycorrhizae, Madras, pp 169–174

Stoyke G, Currah RS (1991) Endophytic fungi from the mycorrhizae of alpine ericoid plants. Can J Bot 69:347–352

Trappe JM (1977) Selection of fungi for ectomycorrhizal inoculation in nurseries. Annu Rev Phytopathol 15:203–222

Trappe JM (1987) Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In Safir G (ed) Ecophysiology of VA mycorrhizal plants. CRC, Boca Raton, Fla., pp 5–25

Trappe JM (1988) Lessons from alpine fungi. Mycologia 80:1–10

Trappe JM, Luoma D (1992) The ties that bind: fungi in ecosystems. In: Carroll G, Wicklow D (eds) The fungal community: its role in the ecosystem, 2nd edn. Dekker, New York, pp 17–27