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# Plant growth and ectomycorrhiza formation by transplants on deglaciated land near Exit Glacier, Alaska

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**Abstract** Ectomycorrhiza (EM) formation on plant roots in successional communities may vary with plant species, plant age, and age of vegetation after disturbance. To evaluate differences in mycorrhizal fungus communities on roots of same-aged seedlings across a deglaciated chronosequence, indoor-grown bioassay plants of four dominant species *Epilobium latifolium* L. (dwarf fireweed), *Populus balsamifera* ssp. *trichocarpa* (Torr.&Gray) Hult. (black cottonwood), *Alnus sinuata* (Regel) Rydb. (Sitka alder), and *Picea sitchensis* (Bong.) Carr. (Sitka spruce) were transplanted into five successional stages (Barren, Isolated Plant, Patchy, Alder, and Cottonwood) following deglaciation near Exit Glacier, Alaska. The species were selected for their successional status and mycorrhiza formation potential [EM or arbuscular mycorrhiza (AM) or both]. Seedlings were transplanted in June 1992, and half were harvested at the ends of the two subsequent growing seasons. The EM communities on *P. balsamifera* differed across the chronosequence while those of the other species did not. Morphotype B dominated the EM on *P. balsamifera* after the Barren stage, and the greatest EM colonization was in the Isolated Plant and Patchy stages. No AM were found. The EM observed on even-aged seedlings in this study were a subset of the EM found on naturally occurring plants (seedlings to mature trees) in a prior study, and some were common to multiple plant species. Most plant growth responses were not significant across stages or were inconsistent among plant species.

**Key words** Ectomycorrhizae · Glacier · Succession · Chronosequence · *Alnus* · *Populus* · *Picea*

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## Introduction

Chronosequences provide opportunities to evaluate mycorrhiza communities on selected plant species across a range of various-aged vegetation communities. Observations of mycorrhizae on naturally occurring plants may indicate what fungus species are present, but bioassays are needed to evaluate mycorrhizae that can form on new seedlings across the seral stages. Differences in ectomycorrhizal (EM) fungus composition have been described on naturally occurring plants at various sites or successional stages (Deacon et al. 1983; Mason et al. 1983; Fleming et al. 1984; Gardner and Malajczuk 1985; Dighton et al. 1986; Last et al. 1987; Gemma and Koske 1990; Cázares 1992; Visser 1995; Helm et al. 1996) but these observations confound plant age with site conditions. Field and greenhouse bioassays have been used to assess the mycorrhiza formation potential of various site treatments or inocula (Moorman and Reeves 1979; Schoenberger and Perry 1982; Miller et al. 1991; Smith et al. 1995). The use of field transplants provides a constant plant age and length of time for mycorrhiza formation across all stages under natural conditions, whereas greenhouse bioassays are conducted under relatively constant, controlled conditions. Field bioassays are essential to determine early EM colonizers when even-aged, naturally occurring seedlings are rare or absent, as occurred in this study.

In an earlier study, we examined the formation of mycorrhizae on roots of naturally occurring *Epilobium latifolium* L. (dwarf fireweed), *Populus balsamifera* ssp. *trichocarpa* (Torr.&Gray) Hult. (black cottonwood), *Alnus sinuata* (Regel) Rydb. (Sitka alder), and *Picea sitchensis* (Bong.) Carr. (Sitka spruce) in six successional stages of a fluvio-glacial chronosequence near Exit Glacier, Kenai Fjords National Park, Alaska (Helm et al. 1996). Substrates ranged from mineral soil to forest floors. Some differences in EM formation by *P. balsamifera* during succession likely resulted from sampling mature trees in late stages and seedlings and saplings in younger stages, as well as changes in soils and environment during succession. This field bioassay was needed to assess EM formation on young, same-aged plants because seedlings of these species were rarely or never found after the Barren stage in this study. One- and 2-year-old field transplanted seedlings should be colonized primarily by early-stage fungi. Some late-stage fungi may also colonize young roots in contact with hyphae of these fungi attached to 'parent' plants (Fleming 1983; Deacon and Fleming 1992).

A field bioassay was performed (1) to compare total EM formation percentage levels among young, similar-aged plants across the chronosequence, (2) to compare EM morphotypes and community composition on seedlings across the chronosequence, and (3) to assess the inoculum density of a site with sparse cover, such as the Barren stage. This would determine whether differences observed between later and earlier stages resulted from tree age or whether these EM communities could form on seedling transplants. Field bioassays such as this include shade, litter depth, soil development, and other factors that change with succession and are absent in greenhouse bioassays.

## Materials and methods

### Study area

Exit Glacier (60° 15' N 149° 30' W) is about 5 km long (1980's) and descends over 700 m from the Harding Ice Field on the Kenai Peninsula, Alaska. The vegetation chronosequence was divided into seven stages: (1) Barren, (2) Isolated Plant, (3) Patchy, (4) Alder, (5) Cottonwood, (6) Spruce-Cottonwood, and (7) Spruce-Hemlock (Helm and Allen 1995). This study focused only on the early successional stages up to the first forested stage (Cottonwood). The most common plant species on the Barren stage were *P. balsamifera* seedlings less than 2 cm tall and *E. latifolium*, especially along drainages. *P. balsamifera* was the dominant plant in the Isolated Plant and Patchy stages, which were relatively open (Helm and Allen 1995). *A. sinuata* dominated the Alder stage, the youngest stage with shade and litter. Mature *P. balsamifera* dominated the Cottonwood forests where the canopy shaded the understory and partially decomposed litter helped form a forest floor. Seedlings <2 years old of the four target species (*E. latifolium*, *P. balsamifera*, *A. sinuata*, and *Picea sitchensis*) were observed only in the Barren stage.

Although there was no meteorological equipment in the Barren stage, it was adjacent to the glacial ice and was noticeably colder and windier than the other stages, especially those with dense vegetative cover, such as the Alder and Cottonwood stages. Mineral soils in the young stages had low levels of nutrients (extractable N, P <1 mg/kg, total N, P <0.10%), while litter layers in the Alder and especially the Cottonwood stages (extractable N 98 mg/kg, extractable P 21 mg/kg, total N 1.58%, total P 0.08%) had greater nutrient concentrations than the three earliest stages (Helm and Allen 1995; Helm et al. 1996). The litter layer in the Alder stand in this study had undecomposed leaves over glacial gravels rather than the partially decomposed leaves characteristic of older stands. The pH values ranged from 8.2 in the Barren stage and 7.2 in the Patchy stage to 5.7 in the mineral soil in the Alder stage and was near 6 through the Cottonwood stage (Helm and Allen 1995).

### Transplant preparation

Seeds were collected when mature during July through October 1991 near Exit Glacier. Seeds of *P. balsamifera* were collected in July when it dispersed, taken to the laboratory, cleaned with forced air, and frozen as soon as possible because of its short viability period (<1 week). Seeds of *E. latifolium* were collected in August and September. Fruits of *A. sinuata* were collected in September and October, taken to the laboratory, air-dried to open the scales, and shaken to remove the seeds. Seeds of *P. sitchensis* were obtained from the Alaska State Forestry Nursery. These seeds had been collected in the Seward area (within 20 km of Exit Glacier) and had been cleaned and dewinged. All seeds were frozen until used.

Seeds were planted in February 1992 in steam-pasteurized glacial till collected from the Exit Glacier outwash plain and placed in Spencer-Lemaire Rootrainers with individual cells 3.7 cm × 4 cm × 11.5 cm deep. We grew the plants indoors until May with an 18-h day length using a combination of metal halide and mercury vapor lights at room temperature (20–25 °C). Plants were watered as needed with tapwater, usually every 2 days, as the water in the large trays holding the Rootrainers dried. Low levels of fertilizer solution were applied at the end of 2 months and/or 3 months to the smaller or less-green plants. The intent of this low-fertility growth regime was to simulate the growing conditions of the outwash plain (except for temperature, moisture, and some nutrients) to produce plants large enough to handle (>2 cm), but not substantially larger or more vigorous than the naturally occurring seedlings in the Barren stage. Plants were hardened outdoors for 2 weeks before transplanting to the site in mid-June 1992, shortly after snowmelt. This resulted in the following mean heights when transplanting: *E. latifolium* 4 cm, *P. balsamifera* 27 cm, *A. sinuata* 5 cm, and *P. sitchensis* 4 cm. The *Populus* was substantially taller than native seedlings, that were generally <2 cm tall in the Barren stage.

Areas were selected in the first five stages (up to the first forested stage), and four blocks containing 20 plants were laid out in a matrix of four rows (one row per plant species) with five plants per row, spaced approximately 2 m apart. Seedlings were planted so that root balls were covered and soils tamped around them. They were watered with glacier stream water when planted. Because Alder stands were small, our transplants were planted around established *A. sinuata* individuals (>2 m tall) rather than in rectangles. Two established *A. sinuata* individuals were selected in each of the four blocks in the Alder stage, and half of the transplants were planted around each established individual in random order. Plants in the three youngest stages with no litter were marked with metal tags nailed into the adjacent glacial till while those in the Alder and Cottonwood stages were marked with both 20-cm wooden stakes and metal tags to facilitate relocation in the litter and to differentiate our transplants from potential colonizers or sprouts in the area.

*E. latifolium* survived poorly indoors so only two seedlings per block per stage were planted. Two additional native plants were taken from naturally occurring patches of *E. latifolium* on the outwash plain and transplanted in each treatment. A subset of these transplants sampled from the field were examined for EM or AM, and no mycorrhizae were found.

### Measurements

Plants were measured for height, horizontal crown length and width (projected surface area), basal diameter, and vigor at the start (June) and end (August) of each growing season and height during mid-season (July). Vigor was estimated as a number from 1 to 5 where 3 was "normal" vigor, 1 was almost dead, and 5 was most vigorous based on plant morphology and greenness. The Isolated Plant community was only sampled at the beginning and end of each season (September) because high, fast water in Exit Glacier Creek during the glacier-melt and rainy seasons of July and August prevented access to this site.

### Root collections and evaluations

Plants from approximately half the surviving individuals were harvested at the end of 1992 (year 1) and the remainder in 1993 (year 2). In the laboratory, depth and width of the extended root system were measured as the plant with its unwashed roots was held upright, roots were washed, and the tops were removed. The root dimensions were intended to give a relative idea of rooting depth and profile width, which may not relate to absorptive area. Roots were stored in 75% ethanol until examination. The tops were oven-dried and weighed for total biomass.

We analyzed the tissue N by flash combustion chromatography using a Carlo–Erba CNS analyzer at the University of California, Riverside. Some individuals were combined within a block because of the small plant size. The plant tissue was ground to a powder with a ball grinder. The plant parts used in the data analysis included all aboveground tissue for *E. latifolium* and *P. sitchensis* and leaves only or aboveground tissue for *P. balsamifera* and *A. sinuata*. Leaves were used for plants with enough tissue, and entire plants were used for very small individuals.

We classified the EM types on roots by the techniques described in Helm et al. (1996) as modified from Agerer (1987–93). This included characteristics of the mantle (color, surface characteristics, sheath patterns), emanating hyphae, and hyphal strands, if present. These descriptions were intended to differentiate EM types but were not intended to be adequate for taxonomic purposes. Each type was given a letter designator and was assumed to represent a fungus species. All root tips were counted and each was assigned to an EM type or nonmycorrhiza category. Some types appeared distinct from others already described, but there was insufficient root material to describe these types. These were lumped in an "Other" category.

Roots of *E. latifolium* were cleared and stained with 10% KOH and Trypan blue by modified methods of Phillips and Hayman (1970) and Kormanik et al. (1980). In earlier studies, we found that 10 min of autoclaving or heating at 90 °C for 1 h caused fragile roots to disintegrate. In this study, depending upon the thickness of these roots, the KOH and roots were gently heated on a hot plate for 0–30 min, then allowed to sit at room temperature until they appeared off-white. Individual root systems cleared at different rates so they were judged on a sample-by-sample basis to determine when the clearing was completed. Stained samples were examined under × 100–400 brightfield optics to find evidence of AM. Some woody roots were also cleared, bleached with $H_2O_2$, stained, and examined for AM. Not all woody roots were analyzed since many tips had already been colonized by EM, thus precluding AM colonization. Furthermore, no evidence of AM had been found on naturally occurring plants and rooted cuttings of *P. balsamifera* we had planted on the outwash plain in other studies (Helm et al. 1996 and personal observation).

### Data analyses

Data comparisons were performed within individual species because of large differences in plant size and mycorrhiza formation. Plant height, crown area (length × width × II/4, assuming ellipsoid shape), root profile area (depth × width/2, assuming triangular profile) were compared across the five stages by one-way analysis of covariance with initial height as a covariate and a blocking factor. Survival and vigor were analyzed by Kruskal-Wallis analysis of variance test for categorical data. Survival during the first year was calculated as the percent of plants alive at the end of the first year relative to the number planted; the survival during the second year was the percent surviving at the end of the second year relative to those alive at the end of the first year that were not harvested. Standard error was reported for survival (categorical) data since usual measures of variation, such as quartiles or ranges, were not useful. The crown and root profile area are not reported because few differences were significant.

Percent colonization for each EM morphotype was calculated for each root system by adding the number of root tips in each EM type, dividing by the total number of root tips, and multiplying by 100. Diversity of mycorrhiza morphotypes was calculated by Simpson's reciprocal diversity index on the mean relative percentage formation within each plant species in each chronosequence stage. Evenness was the diversity index divided by the number of EMF species.

Ectomycorrhiza type data were analyzed by one-way analysis of variance within each year and for each species (*P. balsamifera*, *A. sinuata*, *P. sitchensis*) across the chronosequence to reduce variances. Differences among chronosequence stages were determined by Tukey's HSD test for unequal sample sizes. Multivariate analysis of variance (MANOVA) was used to detect differences in the mycorrhiza communities (composition of EM by morphotype) among successional stages. Plant response data are reported only for the second year since the first year was an establishment year, and EM were just starting to form. The first year (1992) had an unusually short growing season because of late spring snows and cold weather and early freezing temperatures in the fall.

## Results

### Mycorrhiza morphotypes

The main EM morphotypes observed in this study were also found on naturally occurring plant species in this chronosequence (Figs. 1, 2) as described in Helm et al. (1996). The letter names assigned to EM morphotypes in our previous study (Helm et al. 1996) are used here. Single letters designate EM morphotypes believed to be produced by a single fungus species. The legends of Figures 1 and 2 form a list of EM types found on transplanted seedlings of that plant species. Stripes for minor types may not be visible. Some types (e.g. Y, Z) in the establishment year were poorly developed or occurred on only a few tips and may have been early stages of another type. *P. sitchensis* had several possible types that occurred too infrequently to give them names or compare with other types. Types too poorly formed to distinguish were grouped under "Other." Except for *P. sitchensis* during the first year, the "Other" category probably had fewer than 2 or 3 EMF species on each plant species. As noted previously on naturally occurring plants at this site (Helm et al. 1996), hyphal strands and similar structures were relatively rare on these EM morphotypes.

Helm et al. (1996) fully described all morphotypes but brief descriptions are presented here for complete-

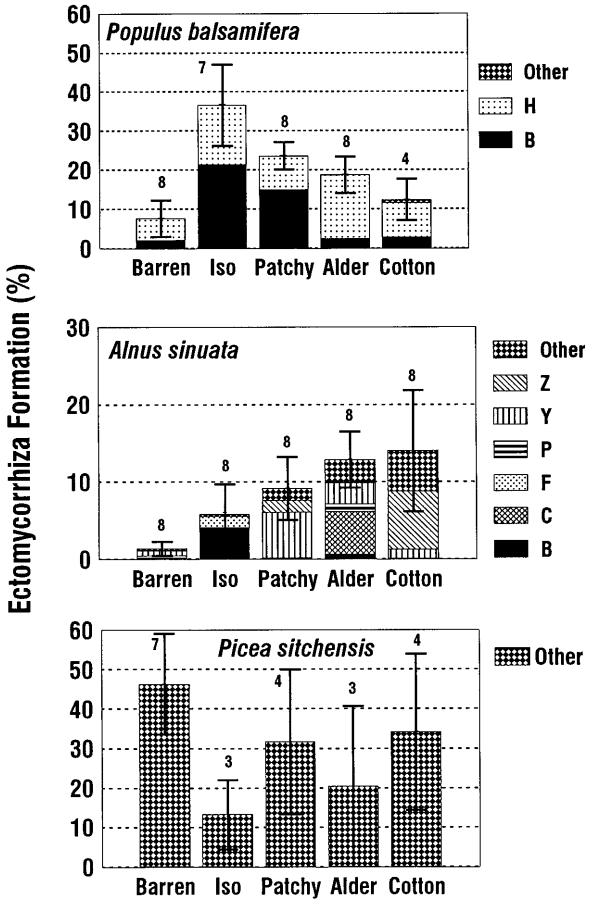


**Fig. 1** Ectomycorrhiza formation (%) by morphotypes (indicated by shading and letters) on first-year seedlings (1992) transplanted into five successional stages on a glacial chronosequence, Exit Glacier. Note different scales on y axes. *Numbers above bars* are number of plants sampled; *bars* 1 SE (*Iso* Isolated Plant stage)

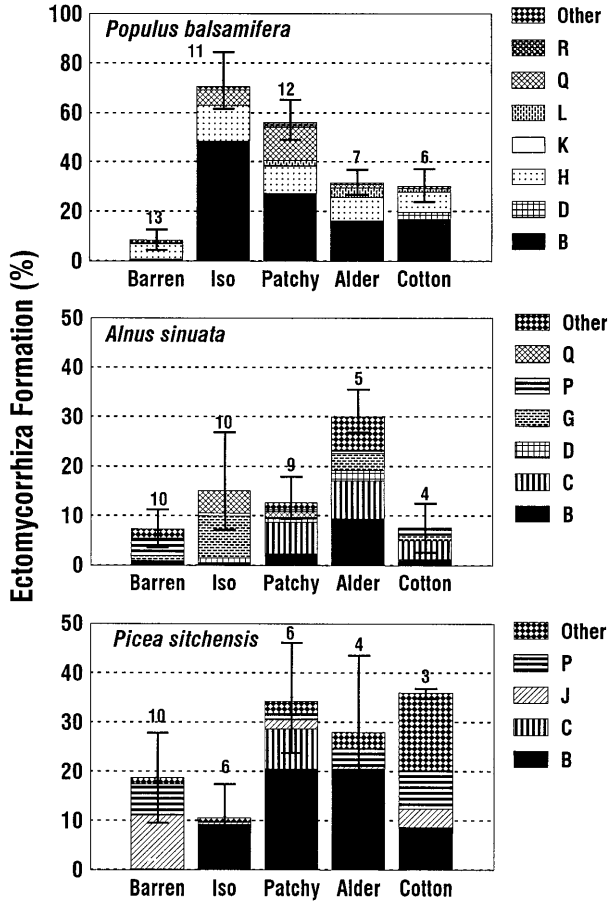


**Fig. 2** Ectomycorrhiza formation (%) by morphotypes (indicated by shading and letters) on second-year seedlings (1993) transplanted into five successional stages on glacial chronosequence, Exit Glacier. Note different scales on y axes. *Numbers above bars* are number of plants sampled; *bars* 1 SE (*Iso* Isolated Plant stage)

ness. Morphotype B was a very dark brown, almost black, type with no observed clamp connections. It was most common on *Salix* and *Populus* individuals, especially in the Isolated Plant and Patchy stages, but less often in the Barren stage (Helm et al. 1996). Type H had a relatively smooth brown mantle, bent or tortuous tips, and many clamp connections. It formed on Salicaceae in the Barren stage, but was less common than type B in later stages. Type G was probably *Alpova diplophloeus* (Zeller) Trappe & A.H. Smith and was found on *A. sinuata*. Type C also occurred mostly on *A. sinuata* but had a long-spiny, dark brown mantle with determinate emanating hyphae with septa and clamp connections. Type Q was a light brown, woolly EM with clamp connections that occurred on naturally occurring and transplanted *Salix*, *Populus*, and *Alnus* (Helm et al. 1996). Type J had a light brown, poorly developed mantle and few emanating hyphae but clamp connections were common. It occurred on *P. sitchensis* in the Barren stage. A lack of fruiting bodies in the early stages prevented identification of most EMF.

## Differences in mycorrhiza formation among stages

No EM or AM were found on *E. latifolium*, so this plant species will not be discussed further.

EM formation on *P. balsamifera* differed among the stages during the first year ($P<0.03$) with greater formation in the Isolated Plant stage than the Barren stage ($P<0.02$) and a gradual nonsignificant decrease from the Isolated stage to the Alder and Cottonwood stages (Fig. 1). Type B was significantly greater in the Isolated and Patchy stages than other stages but type H did not differ among stages ($P>0.67$) during the first year.

Total EM formation by *P. balsamifera* was about twice as great on second-year plants as first-year plants, and the number of identified EM types increased from 2 to 7 (Fig. 2 versus Fig. 1). Plants in the Isolated Plant stage during the second year had significantly greater total EM formation than the Alder and Cottonwood stages as well as the Barren stage ($P<0.05$) (Fig. 2). The EM community composition (multivariate analysis of variance) also differed during the second year

($P<0.001$), but this time B ($P<0.01$), D ($P<0.01$), L ($P<0.05$), Q ($P<0.02$), and R ($P<0.07$) all had univariate differences among stages. Morphotype H was the major type in the Barren stage while type B dominated the Isolated and Patchy stages, the same as with naturally occurring plants (Helm et al. 1996). The relative percentage EM formed by type B also increased relative to type H between the first and second years except in the Barren stage. Diversity of EM types tended to be similar across the chronosequence, with Simpson's reciprocal index varying from 2 to 2.7, richness varying from 5 to 7, and evenness varying from 0.34 to 0.53 (Fig. 3).

The total EM formation on *A. sinuata* did not differ significantly among stages during either the first year ($P>0.29$) (Fig. 1) or the second ($P>0.22$) (Fig. 2). Although the EM fungus community composition did not differ among stages ($P>0.19$), each stage tended to be dominated by a different type.

During the second year, the EM community composition on *A. sinuata* varied across the chronosequence ($P<0.01$) (multivariate), largely influenced by the occurrence of types B ($P<0.04$) and C ($P<0.14$) (univariate) in the Alder stage. *A. sinuata* had the greatest diversity in the Alder stage with a Simpson's index of 4.7

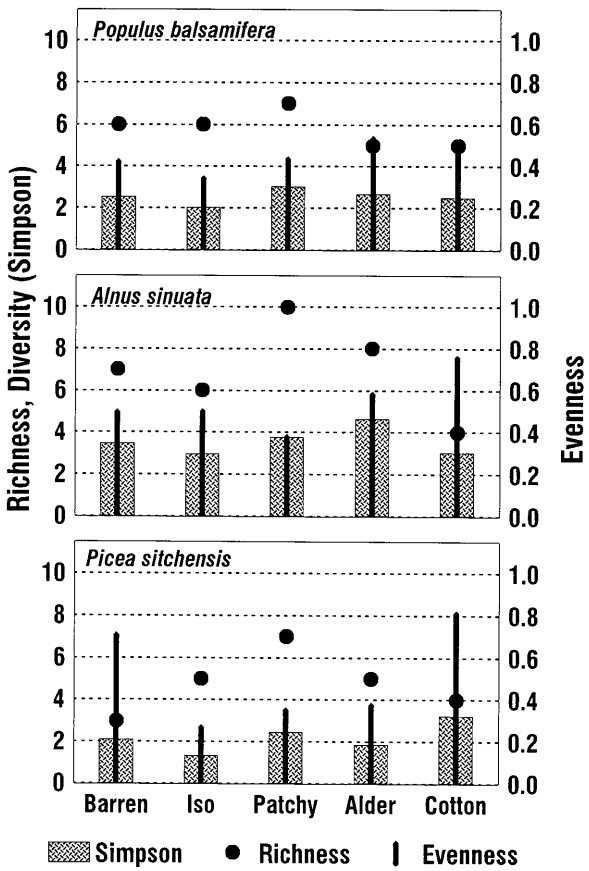


**Fig. 3** Simpson's diversity, richness, and evenness of ectomycorrhizae on second-year (1993) seedlings transplanted into five successional stages on a glacial chronosequence, Exit Glacier (*Iso* Isolated Plant stage)

(Fig. 3). The fewest types described were in the Cottonwood stage (4), although one or two more types may have been present in the "Other" category.

EM formation by *P. sitchensis* was quite variable during both years, especially the first. Some individuals were heavily colonized and others were not, resulting in no significant differences across the stages (year 1, $P>0.65$; year 2 $P>0.41$) (Figs. 1, 2). Small samples the first year had few root tips on each plant, which made distinguishing types or descriptions extremely difficult.

The EM community composition on *P. sitchensis* differed across stages in year 2 ($P<0.08$), partly as a result of type B being important in all stages except the Barren. In addition, several infrequent types were grouped under "Other" in the Cottonwood stage.

### Plant responses

The 2-year survival of unharvested plants occurred for *P. balsamifera* and was best in the three earliest stages and lowest in shaded understories of Alder and Cottonwood stages ($P<0.01$) (Table 1), where shade intolerant *Populus* would not normally grow (Helm and Allen 1995; Helm and Collins 1997). Most plants of all species survived the first growing season, but the overwinter and second growing season survival were substantially lower, especially in the Alder and Cottonwood stages. Litter covered many small seedlings, making survival difficult. Vigor of surviving plants did not differ for stages ($P>0.22$) or species ($P>0.13$) (Table 1).

Transplants of *P. balsamifera* had similar heights ($P>0.61$) and aboveground biomass ($P>0.54$) (Table 1) across the five stages by the end of the 2-year study. However, the tissue N concentration was greatest in the Alder and Cottonwood stages ($P<0.02$) (Table 1). Plant responses, which were generally not significant across the chronosequence, appeared more related to other factors such as shade than to EM formation, which did differ across the chronosequence.

Transplanted seedlings of *A. sinuata* were tallest in the Cottonwood stage ($P<0.02$), although aboveground biomass did not increase ($P>0.35$) (Table 1). Tissue N concentrations were highly variable (Table 1).

The lowest biomass of *P. sitchensis* ($P<0.08$) occurred in the Cottonwood stage, although height did not differ across the stages ($P>0.50$) (Table 1). Biomass was almost twice as great in the Barren stage as in the Cottonwood stage. Tissue N did not differ significantly across the stages ($P>0.13$).

## Discussion

### Comparisons with ectomycorrhizae on naturally-occurring plants

The EM observed on plant roots in this study are those that can form on roots of seedlings within two growing

**Table 1** Growth and survival values for transplanted plant species by stage after are growing season

| Species and stage | | n | Height (cm) | | Aboveground biomass (g/plant) | | Vigor[a] | Survival (%) | | Nitrogen (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SE | Mean | SE | Mean | Mean | SE | Mean | SE |
| *Populus balsamifera* | | | | | | | | | | | |
| | Barren | 12 | 28.1 | 2.2 | 1.10 | 0.17 | 3 | 100 | 0 | 0.6738 | 0.0536 |
| | Isolated | 11 | 26.9 | 2.9 | 0.97 | 0.34 | 3 | 100 | 0 | 0.6263 | 0.0893 |
| | Patchy | 12 | 31.5 | 3.3 | 1.37 | 0.25 | 3 | 100 | 0 | 1.0536 | 0.0989 |
| | Alder | 6 | 27.2 | 6.0 | 1.19 | 0.36 | 3 | 67 | 16 | 2.2796 | 0.1887 |
| | Cottonwood | 5 | 29.1 | 2.8 | 0.66 | 0.13 | 2 | 71 | 18 | 2.0968 | 0.2988 |
| *Alnus sinuata* | | | | | | | | | | | |
| | Barren | 7 | 3.3 | 0.6 | 0.07 | 0.02 | 2 | 64 | 15 | | |
| | Isolated | 9 | 4.1 | 0.4 | 0.06 | 0.02 | 2 | 67 | 14 | 1.7334 | 0.1424 |
| | Patchy | 7 | 3.6 | 0.4 | 0.08 | 0.02 | 2 | 64 | 15 | 1.4777 | 0.8055 |
| | Alder | 5 | 5.3 | 0.8 | 0.07 | 0.01 | 2 | 56 | 17 | 1.5012 | 0.3038 |
| | Cottonwood | 3 | 7.6 | 1.6 | 0.10 | 0.02 | 3 | 57 | 20 | 2.3692 | 0.9013 |
| *Picea sitchensis* | | | | | | | | | | | |
| | Barren | 7 | 3.3 | 0.3 | 0.08 | 0.02 | 2 | 78 | 14 | 0.7339 | 0.0231 |
| | Isolated | 6 | 3.6 | 0.4 | 0.04 | 0.01 | 2 | 67 | 21 | 1.1010 | 0.2337 |
| | Patchy | 4 | 4.0 | 0.6 | 0.09 | 0.02 | 3 | 67 | 21 | 0.9426 | 0.0774 |
| | Alder | 4 | 4.8 | 1.1 | 0.08 | 0.04 | 3 | 50 | 19 | 1.0029 | 0.0817 |
| | Cottonwood | 3 | 4.2 | 1.4 | 0.04 | 0.02 | 4 | 43 | 20 | 1.2278 | 0.0000 |

[a] Vigor is a number between 1 and 5 with 1 being almost dead, 3 normal, and 5 very vigorous. This parameter is based on greenness, leafiness, and general robustness of plant

seasons and may differ from those on naturally occurring plants with ages of 1–60 years or more for the stages in this study or several hundred years in older stages. Overall, the EM formation on transplants during the first 2 years was lower and less diverse (richness plus evenness) than on naturally occurring plants in this same chronosequence (Helm et al. 1996). In particular, the EM formation on 2-year-old transplants in the Barren stage ranged from only 5 to 20%, whereas it averaged 25–35% on naturally occurring plants (Helm et al. 1996). However, the naturally occurring seedlings in the Barren stage were slightly older (3–5 years) and thus had a longer time for EM to form.

The occurrence of some EM morphotypes on multiple plant species on the seedlings of this study as well as the naturally occurring plants of the earlier study (Helm et al. 1996) suggests that EMF may be shared among plant species. This sharing of EMF among hosts could facilitate establishment of seedlings in the root zone of older plants. For instance, type B was very common on established *P. balsamifera* in Isolated Plant, Patchy, and Alder stages (Helm et al. 1996). It was also found on transplanted seedlings in this study, especially on the *P. balsamifera* and *P. sitchensis*. Hence, established *Populus* may be able to facilitate establishment of *Picea* and subsequent community development on cold, low nutrient soils by sharing EMF. Environmental factors also undoubtedly play a role.

The percent mycorrhiza formation on transplanted seedlings of *P. balsamifera* decreased in the Alder and Cottonwood stages compared with Isolated and Patchy stages. In contrast, naturally occurring plants had the least colonization in the three earliest stages (Helm et al. 1996). The decrease may have resulted from shading, which would have reduced photosynthesis. Mature *P. balsamifera* in the Cottonwood stage had high EM diversity that was not present on seedling transplants (Helm et al. 1996). Seedlings of *P. balsamifera* are shade intolerant, and naturally occurring seedlings were not found in the Alder or Cottonwood stages. The simpler EM communities on the transplants may result from the reduced time for development, suggesting that many EM types on naturally occurring plants needed more time to form, a possible indication of late-stage EM fungi.

Total EM formation by *P. balsamifera* was lowest in the Barren stage as it was with natural plants (Helm et al. 1996). Type B was the predominant EM type on naturally occurring plants in most non-forested stages (Isolated Plant through Alder stages) (Helm et al. 1996) and was a major EM type in all stages beyond the Barren stage. This is consistent with previous suggestions that type B is limited by dispersal, especially in the Barren stage, but forms EM readily on *P. balsamifera* in older stages closer to inoculum sources (Helm et al. 1996). Type B averaged less than 20% relative colonization on naturally occurring plants in the Cottonwood stage but about 50% on transplanted seedlings in the same stage. This suggests that type B is formed by an early-stage fungus. Types B and H have the same inverse relationship on these transplants as they did on naturally occurring plants (Helm et al. 1996).

EM formation percentages by *A. sinuata* did not differ across the five stages either in the bioassay (Figs. 1,

2) or on naturally occurring plants (Helm et al. 1996). This was consistent with observations by Miller et al. (1991) that many EM fungi on *Alnus* are found across many habitats, even though they tend to be host specific (Molina 1979; Molina et al. 1992). Types G (probably *Alpova diplophloeus*) and C were important on *A. sinuata* transplants as well as on naturally occurring plants (Helm et al. 1996). *Alpova* was also reported on *Alnus* in floodplain and upland sites in interior Alaska (Brunner et al. 1992).

Type B was a major component on bioassay seedlings of *P. sitchensis* in all stages except the Barren, but was rare on naturally occurring *P. sitchensis* in these stages (Helm et al. 1996). This may be an artifact of small sample sizes in both studies, especially among the naturally occurring plants, where few *Picea* plants were available for sampling, or it could be a greenhouse contaminant. Data from other forest communities (Helm et al. 1996 and unpublished data) suggest that diversity, especially evenness, increases in late stages. EM formation by *P. sitchensis* in the Cottonwood stage was substantially greater than by *P. balsamifera* and *A. sinuata*, possibly because *P. sitchensis* is shade tolerant and is found in these understories naturally.

Many EM morphotypes (10 plus several lumped under "Other") were found on naturally occurring *P. sitchensis* in forested stages (Helm et al. 1996), but relatively few (4 plus "Other") were found on 2-year-old transplants (Fig. 2). This suggests that some EMF formed by mature trees in later stages of succession either do not colonize plants in earlier stages or require more time to form than the 2 years of the study. Differences in forested and early succession EMF species could explain why the EM on *P. sitchensis* transplants (>2 years old) lost their mantles (formed in the greenhouse and nursery prior to transplanting on outwash plain) 3 years after being transplanted to the early stage site in the study by Chapin et al. (1994).

## Plant responses

Plant growth and mycorrhiza formation percentage or EM types were seldom related. For instance, the EM formation percentage by *P. balsamifera* varied across stages with a decrease in the Alder and Cottonwood stage, but most plant responses did not differ across the stages. Survival of *P. balsamifera* overall may have been better than that of other species because the transplants were much taller than the other species to start (27 cm versus 4–5 cm) and would be less affected by litter.

Plants were generally least vigorous in the Cottonwood stage and had formed fewer EM than in earlier stages. This suggests that factors associated with succession (shade, litter) limited growth, and is consistent with observations on *P. sitchensis* (Chapin et al. 1994), where shading and interactions with established plants limited growth of seedlings in established plant communities.

Shade may have reduced growth, EM formation, and nodulation of *A. sinuata* in the Alder and Cottonwood stages where many seedlings were etiolated, had poorly formed root systems, and lacked sheaths and nodules. We saw no naturally occurring seedlings in these stages for comparison, but naturally occurring seedlings in the Barren stage had EM and nodules (Helm et al. 1996).

*A. sinuata* can facilitate establishment of *P. sitchensis* in the open but competes with it if *Alnus* establishes first (Chapin et al. 1994). In our bioassay, *Picea* was not larger in the Alder stage than in earlier stages, consistent with this prior result. Our Alder stage was not well developed, and the litter layer consisted primarily of recent deposition of relatively undecomposed leaves. Soil conditions were closer to the mineral soils of Patchy stage than to a well-developed Alder stand, which was not available at Exit Glacier (Helm and Allen 1995).

This study confirmed observations on naturally occurring plants that EM formation in the Barren stage is low. This may result from low inoculum density or low organic matter and nutrient content in the soil, although it could also result from slow rates of mycelium growth and EM formation in the cold, windy environment of the Barren stage.

This field bioassay was useful to compare EM formation on same-aged plants across a chronosequence with different environmental conditions at each stage, especially where naturally occurring seedlings were not found. General EM formation patterns on same-aged seedlings followed the same general patterns observed on established plants. Plant growth was highly variable and appeared more related to local environment factors such as soil fertility, shade, or litter depth than to total percentage EM colonization or species composition of EM. However, some EMF were observed on multiple plant species and potentially could be shared among established hosts and new seedlings. This potential to share EMF among plant species may be more important than plant growth responses for community development in primary succession and needs to be explored further.

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