Mycorrhiza (2009) 20:51–66
DOI 10.1007/s00572-009-0263-0

ORIGINAL PAPER

Effects of growth medium, nutrients, water, and aeration on mycorrhization and biomass allocation of greenhouse-grown interior Douglas-fir seedlings

Olga Kazantseva · Marcus Bingham · Suzanne W. Simard · Shannon M. Berch

Received: 21 January 2009 / Accepted: 12 June 2009 / Published online: 2 July 2009

Abstract Commercial nursery practices usually fail to promote mycorrhization of interior Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca* (Beissn.) Franco] seedlings in British Columbia, which may account for their poor performance following planting in the field. We tested the effects of four nursery cultivation factors (nitrogen fertilization, phosphorus fertilization, watering, and soil aeration) and field soil addition on mycorrhization, survival, growth, and biomass allocation of interior Douglas-fir seedlings in a series of greenhouse experiments. Where field soil was added to the growing medium, mycorrhization and root/shoot ratios were maximized at lower levels of mineral nutrient application and aeration. Where field soil was not added, mycorrhization was negligible across all fertilization and aeration treatments, but root/shoot ratio was maximized at lower levels of mineral nutrients and the highest level of aeration. Regardless of whether field soil was added, intermediate levels of soil water resulted in the best mycorrhizal colonization and root/shoot ratios. However, field soil addition reduced seedling mortality at the two lowest water levels. A cluster analysis placed ectomycorrhizal morphotypes into three groups (*Mycelium radicis-atrovirens* Melin, *Wilcoxina*, and mixed) based on their treatment response, with all but two morphotypes in the mixed group whose abundance was maximized under conditions common to advanced seedling establishment. For maximal mycorrhization and root development of interior Douglas-fir seedlings, nurseries should minimize addition of nitrogen and phosphorus nutrients, maximize aeration, provide water at moderate rates, and, where possible, add small amounts of field soil to the growing medium.

Keywords Reforestation · Ectomycorrhizas · Nursery · Seedlings · Fertilizer · Watering · Aeration · Douglas-fir · Dark septate endophyte

O. Kazantseva
Canadian Food Inspection Agency,
Vancouver, BC, Canada

M. Bingham · S. W. Simard (✉)
Department of Forest Sciences, University of British Columbia,
Vancouver, BC, Canada V6T 1Z4
e-mail: suzanne.simard@ubc.ca

S. M. Berch
British Columbia Ministry of Forests & Range,
Victoria, BC, Canada V8W 9C4

Introduction

Many woody plants are dependent on ectomycorrhizal (ECM) fungi for their growth and survival (Stack and Sinclair 1975; Perry et al. 1989; Horton et al. 1999; Nara 2006). Mycorrhizas usually incur the greatest benefit under conditions of high stress (Dickie et al. 2002, 2005; Dickie and Reich 2005), principally those where soil nutrients are limiting (Treseder and Allen 2002; Hoeksema and Schwartz 2003; Jones and Smith 2004; Johnson et al. 2006), depending upon the plant and fungal species involved (Johnson et al. 1997; Klironomos 2003; Jones and Smith 2004). Conifers are difficult to establish where climatic stresses such as summer drought, summer frost, and harsh winters are the norm, as is the case in the dry interior Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca* (Beissn.) Franco] forests of British Columbia (Simard et al. 2003; Heineman et al. 2003). Root development and ECM colonization are especially important in these environments for facilitating water and nutrient uptake (Jones et al. 2003). To survive the first season, planted nursery seedlings must overcome transplant shock,

establish root–soil contact, and commence water and nutrient uptake rapidly through abundant ECM mycelia (Perry and Amaranthus 1997). However, nursery practices, including frequent addition of fertilizers and water, may be inappropriate for mycorrhization of container-grown conifer seedlings. Interior Douglas-fir grown in British Columbia nurseries, in particular, is usually non-mycorrhizal prior to outplanting (Berch et al. 1999).

Environmental factors influence the mycorrhizal symbiosis, which in turn influences plant establishment, growth, and fitness (Koide 1991; Hartnett and Wilson 2002; Duñabeitia et al. 2004; Rillig 2004). The importance of mycorrhizal fungi to seedling establishment has been studied in a number of conifer species (Horton et al. 1998; Niemi et al. 2004; Teste et al. 2004), but few studies have assessed how mycorrhizal effects on seedling establishment change with soil conditions. In dry Douglas-fir forests, reduced mycorrhizal diversity and abundance have been associated with reduced survival and growth of newly planted conifer seedlings (Simard et al. 2003). Douglas-fir seedlings that are non-mycorrhizal at the time of planting can remain low in ECM colonization and richness (commonly dominated by *Wilcoxina rehmii*) for the first year post-planting, and those that do not readily form ECM or link into the mycorrhizal network of existing trees can suffer reduced establishment success (Teste and Simard 2008). Douglas-fir establishment and growth have been more successful in climates where water stress is lower (Simard et al. 2005; Greisbauer 2008) and where planted seedlings are rapidly colonized by a diverse fungal community (Jones et al. 1997), with clear patterns of ECM succession to 100 years post-disturbance (Twieg et al. 2007). Improving mycorrhization of interior Douglas-fir seedlings in the nursery may increase planting success and aid in the recovery of dry Douglas-fir ecosystems after disturbance.

Most commercial nursery treatments aim to maximize seedling shoot and total growth under the assumption that these are the most important factors for establishment and competitive success in the field (Hunt 1992). These seedlings are poorly conditioned, however, for sites where establishment is limited more by access to water and soil nutrients than competition for light (Simard et al. 2003). In the dry Douglas-fir forests of British Columbia, establishment success of nursery-grown Douglas-fir seedlings is commonly less than 70% (Newsome et al. 1991). Nursery cultures that maximize root-to-shoot ratio (R/S), the number of short root tips, or ECM colonization may improve establishment success (Chapman 1991). Root and mycorrhizal development in nursery stock depends on many factors that can be controlled in the nursery, including the structure, nutrition, pH, moisture, temperature, and aeration of the growing medium. Commercially available biological inoculants have been used to influence the mycorrhization of Douglas-fir (Berch et al. 1999), but these are expensive and do not necessarily contain the best fungi for facilitating establishment on local sites. Specific nursery practices and materials that can be adjusted to influence the architecture, volume, and mycorrhization of the root system include the fertilizer and watering regime, growing medium, container design, cavity size, copper treatment, and use of plant growth regulators or other biostimulants (Hunt 1992; Campbell et al. 2003). These factors can be tested and adjusted for optimal mycorrhization and root morphology for high planting survival and growth at minimal cost.

The overall objective of this study was to identify the most favorable nursery practices leading to advanced mycorrhization (based on colonization and morphotype composition that develop as seedlings establish) and rooting structure of containerized nursery-grown interior Douglas-fir seedlings and to relate the responses to seedling survival and growth. To meet this objective, we tested the effects of field soil addition crossed with four nursery cultivation practices (watering, aeration, nitrogen fertilization, and phosphorus fertilization) on mycorrhization, growth, and biomass allocation patterns of interior Douglas-fir in a series of greenhouse experiments.

Materials and methods

Experimental design

We conducted four factorial experiments where different nursery cultural practices were systematically varied with and without forest soil addition. All four experiments used a 2×5 factorial set of treatments where the first factor was soil addition (two levels: with or without field soil added) and the second factor was either soil water, aeration, nitrogen (N) fertilization, or phosphorus (P) fertilization (five levels each). Treatments 1–5 for each experiment had no field soil added and treatments 6–10 were the same cultural treatments but with field soil added, for a total of ten treatments. Each treatment was replicated 12 times. Each replicate contained 18 trees in a single styroblock. The total number of seedlings in each experiment was 2,160. Treatments were organized as a completely randomized design, with replicate styroblocks moved daily and re-randomized weekly.

Treatments

Aeration experiment Aeration was manipulated by adding Styrolite® to peat moss; 100% peat moss is the standard medium used by most commercial nurseries. The following aeration treatments were applied: (A1) 100% peat moss, (A2) 90% peat moss + 10% Styrolite®, (A3) 80% peat moss + 20% Styrolite®, (A4) 70% peat moss + 30%

Styrolite®, and (A5) 60% peat moss + 40% Styrolite®. Increasing Styrolite® content from 0% to 40% increased aeration porosity from 6.4% to 19.7% in the basic medium with no field soil added and from 4.3% to 15.1% where field soil was added. In both soil addition treatments, increased aeration porosity with Styrolite® addition corresponded with a decline in total porosity by 7–12% and a decline in water holding porosity by 11–13%. The seedlings in this experiment were harvested 29 weeks after sowing.

Watering experiment The watering treatments were based on the percent of irrigation weight recommended by the nurseries. When designing the watering treatments, we took into consideration that Douglas-fir is very sensitive to over-irrigation and can produce excessive top growth at the expense of caliper and root growth under high water levels (Hunt 1992). Water treatment levels were calculated as a percentage of the container capacity (CC), which is the amount of water in the block after full saturation and drainage. The following water levels were applied: (M1) 100% CC, which is equivalent to field capacity, (M2) 5% of CC, (M3) 10% of CC, (M4) 20% of CC, and (M5) 30% of CC. The seedlings were harvested 21 weeks after sowing.

Nitrogen and phosphorus fertilizer experiments The production of ECM plants requires extra care in formulating fertilizer regimes and fertilizer rates (Hunt 1992). High rates of fertilizer often suppress mycorrhiza development of container seedlings (Marx et al. 1977). The reduction in fertility, however, may delay shoot development and the achievement of height and root collar diameter targets. These experiments were therefore carefully designed based on common nursery operational rates in British Columbia. Most nurseries target good shoot performance, which does not necessarily result in a good root system with abundant ectomycorrhizas. In general, nursery managers use a wide range of N levels during each growth stage, but the common trend is toward moderate N during the establishment phase, higher levels during the rapid growth stage, and low N during the hardening phase. Some nurseries keep N/P ratios steady during these two phases of growth, but most nurseries change them considerably (Hunt 1992).

In our experiments, we varied N or P gradually while keeping the other nutrients at standard nursery levels (Table 1). As a result, N:P varied from 0.5:1 to 20:1 during the establishment stage and from 1.5:1 to 20:1 during the rapid growth stage. The N experiment was harvested after 23 weeks and the P experiment after 27 weeks.

Greenhouse conditions

The four experiments were conducted in a climate-controlled greenhouse at the University of British Columbia. We applied standard cultural treatments used in British Columbia conifer container nurseries. Seedlings were grown in standard 415B Styroblock® containers that were cut into smaller blocks (3×6=18 cavities for each) for more efficient use of the greenhouse bench space. Each cut Styroblock® was equivalent to a single replicate (or experimental unit) in every experiment.

The Styroblock® cavities were 14.9 cm in depth, 3.6 cm in diameter (at the top), and had a cavity volume of 108 ml. Containers were filled with growing media and sown manually. The following mixture comprised the basic growing medium: 0.5 m^3 Rich Grow Peat® moss, 0.5 m^3 Sun Grow Peat® moss, 1.0 kg dolomite, 1.0 kg gypsum, 0.1 m^3 Styrolite®, and 77 l of H_2O. As described above, 0.1 m^3 field soil (~10% by volume) was added to half of the treatments. The mineral soil was collected from a single forested site near Malakwa, British Columbia, which is located in the Thompson moist, warm variant of the Interior Cedar Hemlock biogeoclimatic zone (Lloyd et al. 1990). The dominant tree species in the 120-year-old forest were *Pseudotsuga menziesii*, *Betula papyrifera* (Marsh.), *Thuja*

Table 1 Nitrogen and P treatment levels in the N and P experiments

Experiment	Treatment symbol		Nutrient levels (ppm)	
	Without field soil	With field soil	Establishment stage	Rapid growth stage
Nitrogen	N1	N6	N10 P20 K80	N30 P20 K80
	N2	N7	N20 P20 K80	N60 P20 K80
	N3	N8	N40 P20 K80	N80 P20 K80
	N4	**N9**	**N60 P20 K80**	**N100 P20 K80**
	N5	N10	N100 P20 K80	N120 P20 K80
Phosphorus	P1	P6	N100 P5 K80	N100 P5 K80
	P2	P7	N100 P10 K80	N100 P10 K80
	P3	P8	N100 P15 K80	N100 P30 K80
	P4	P9	N100 P25 K80	N100 P40 K80
	P5	P10	N100 P60 K80	N100 P50 K80

Bold typed numbers represent standard nursery levels of nutrients

plicata D., and *Tsuga heterophylla* (Raf.) Sarg. The mineral soil was a sandy loam Humo-Ferric Podzol overlain by a moder forest floor and underlain by glacial fluvial parent material. Forest floor was removed and mineral soil was collected to 20-cm depth from ten randomly selected points. These subsamples were bulked into a single sample, sieved to 2 mm, and stored for 2 days at 4°C prior to experiment assembly.

Interior Douglas-fir seeds (seedlot 48520) were stratified at the BC Ministry of Forests and Range Tree Seed Centre. Upon arrival, the seeds were soaked for 2–4 h in 3% hydrogen peroxide solution to reduce the possibility of seedborne *Fusarium* infection. Two stratified seeds were sown per cavity and were covered with a protective 5-mm layer of sterilized sand. Seeds were sown and watered between October 18 and 20, 2003. To grow seedlings through the winter with growth rates similar to a spring-sown crop, we used high-pressure sodium lights.

During the tree germination and growth stages, we adhered to the climatic regimes recommended by Kolotelo et al. (2001). During stage 1, from sowing to emergence from the growing medium, temperatures were maintained between 21°C and 25°C without a day/night difference, and relative humidity was maintained around 95% using overhead misting. The media were kept moist to wet, but not saturated, to maintain aeration porosity. Supplemental lighting was used and the photoperiod was equal to 20 h. Air circulation was minimal during this stage. During stage 2, from emergence from the growing medium to the first true leaf emergence, the same temperature range was maintained as for stage 1, but the day/night regime differed depending on the photoperiod. Light intensity was approximately 50% of full sun. Humidity was kept in the range of 85–95% and air circulation was increased. Seed coats were kept moist until they shed; moisture was determined by visual observation and by touch. If seed coats slid off easily from the seed ends, no misting was required. If seed coats stuck to the needle ends, a low volume misting was applied. After full germination was achieved, the trees were thinned to one seedling per cavity. Empty cavities were transplanted from other cavities that had two germinants. Seedlings were fertilized with 30 ppm N 2 weeks after germination. During stage 3, from establishment to the rapid growth stage, target daytime growing temperature was kept in the range of 18–21°C. Relative humidity was maintained at 60–70%. Fertilization was dropped to half rate irrespective of seed coat drop or at sign of secondary needle emergence.

Treatment application methods

For applying the aeration and watering treatments, we first conducted a pilot study to determine CC for media formulations and the water volume required to reach the treatment soil water contents. Total porosity, aeration porosity, and water holding porosity were determined for all ten media mixtures used in the experiments, as well as how they correspond to the additions of various volumes of Styrolite® and field soil.

To determine when irrigation was required for any experiment, three blocks in every treatment were monitored daily by weighing to determine when the moisture level had dropped below the treatment level. In the aeration, N and P experiments, seedlings were watered each time to full saturation. In the water experiment, by contrast, we applied comparatively small amounts of water. Consequently, every Styroblock® was weighed and irrigated separately.

For the fertilizer treatments, we used fertigation rather than incorporating controlled release fertilizers (CRFs) into growing media because CRFs would have released too much fertilizer over the 4- to 5-month period of our experiments. From weeks 1 to 10 (stages 1 and 2), we applied Peters Conifer Starter® (7-40-17). After week 10 (stage 3), we applied Conifer Grower® (20-7-19) according to the experimental design (Table 1). Some macronutrients were added to complete our target nutrient concentrations. Fertigations were applied in the morning to let the leaves dry before night for the prevention of fungal diseases.

Measurements

At the time of harvest, six trees from each block (the middle row only, to exclude edge effects) were evaluated for height, root collar diameter, and then harvested for root and shoot biomass (dry weight). Roots and shoots were separated at the root collar and adhering medium was washed from the roots. Root and shoot tissues were dried at 70°C and weighed separately.

Root collar diameter was not measured in the watering experiment because the seedlings did not reach full size in some of the treatments. Many were gradually dying or barely surviving. We quantified mortality patterns over the period of the watering experiment.

Upon harvest of each experiment, two seedlings were randomly selected from each experimental unit for ECM morphotyping (total number of seedlings=2 seedlings per Styroblock® X 12 blocks/treatment × 10 treatments × 4 experiments=960 seedlings). The shoots were removed and the root plugs were placed in plastic bags in cold storage for later examination. When morphotyping was conducted (within 2 months of harvest), the root systems were gently washed, cut into 2-cm fragments, and placed in a glass tray with a 2.5-cm grid. One hundred live root tips were randomly selected, examined at 100× and 400×, and then placed into categories based on morphology. Each morphotype was described in detail, including mycorrhizal color and branching pattern; mantle color and pattern;

presence, shape, and size of cystidia; presence of external hyphae; and presence of rhizomorphs. The morphotypes were compared with published descriptions of ectomycorrhizas formed by known mycobionts (e.g., Agerer 1987–1998; Ingleby et al. 1990; Goodman et al. 1996).

Data analysis

In experiments examining cultural practice effects on biotic community structure and function, functional groupings of organisms are often more conceptually useful in analyses than are individual taxa. The use of functional groupings has become commonplace in applied plant community research and, in principle, should be useful when applied to ECM fungi. Our approach categorizes fungal symbionts into groups based on their prevalence among treatments (representing environments differing in functional and adaptational niches) and then assesses how the groups respond to various environmental conditions and thus could be viewed as a community analysis in the context of a set of experiments. Fungal symbionts were grouped using cluster analyses following the approach of Levang-Brilz and Biondini's (2002) for plants, but ECM fungal morphotype colonization in response to the inoculation treatments was used here in place of intrinsic traits of the organisms in question. The following statistical analyses use the proportion of root tips colonized by these groups in place of individual taxa. For details of the cluster analysis methodology, see Levang-Brilz and Biondini (2002). We employed the group average hierarchical linkage method rather than Ward's (McCune and Grace 2002), as Ward's resulted in a highly collapsed structure. Groups were separated using a 50% cutoff of the maximum Euclidean distance (Levang-Brilz and Biondini 2002).

Data were analyzed using the SAS System for Windows, V8 (2004). All variables in each experiment were analyzed using a two-way factorial set of treatments in a completely randomized design using the mixed procedure. Colonization counts were completed for only the median and extreme levels in the watering experiment; thus, only three moisture levels were used in the subsequent analyses. Data for proportion of root tips colonized were arcsine-square-root-transformed prior to statistical tests. All other variables passed tests for normality and equality of variance prior to analysis. Means were separated using Tukey–Kramer multiple comparison tests (α=0.05).

Results

Morphotype groups

Where field soil was not added, mycorrhization was reduced across all treatments (total colonization of 41% for non-enriched media versus 66% for soil-enriched media) and two morphotypes were absent (*Rhizopogon* and an unknown); thus, the cluster analysis and all fungal community analyses were only performed on the field soil treatments. The cluster analysis resulted in three groups of ECM morphotypes (p<0.0001, Fig. 1), and these groups were used to test for treatment effects on percent colonization. Group 1 comprised solely of dark septate endophytes

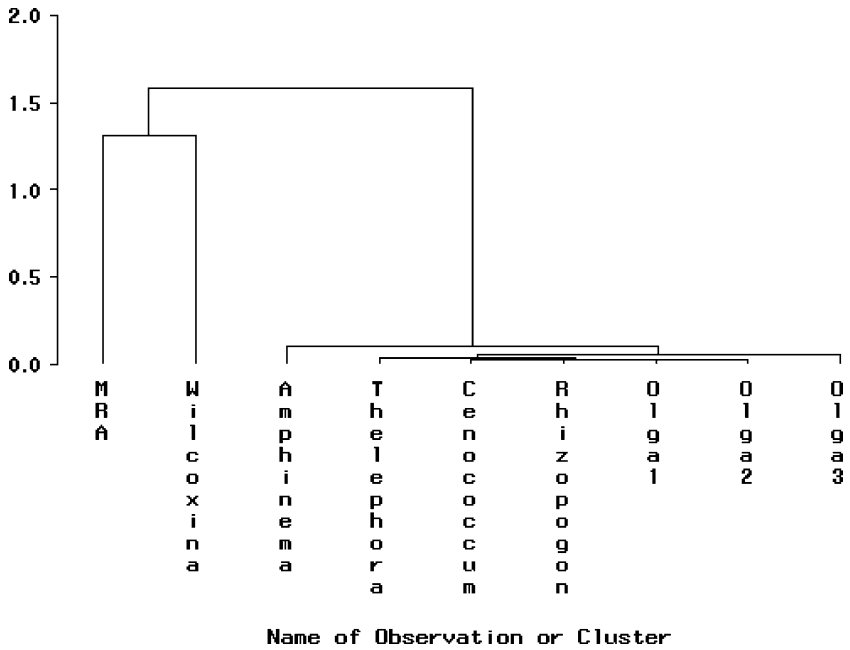

Fig. 1 Average method cluster analysis with Euclidean distance measure results for the ectomycorrhizal morphotypes found on Douglas-fir seedlings. Results are based on data from all experiments. The clusters were significant at p<0.0001

(DSE; formerly known as *Mycelium radicis-atrovirens* Melin; Mandyam and Jumpponen 2005); group 2 comprised *Wilcoxina* spp. (formerly known as E-strain; Yang and Korf 1985); and group 3 comprised *Amphinema* spp., *Cenococcum* spp., *Rhizopogon* spp., *Thelephora* spp., and three unknown morphotypes. Percent colonization by the three groups differed among field soil treatments in all experiments ($p<0.05$, Table 2) except at the highest moisture level in the watering experiment where groups differed significantly using the Pearson's type III sum of squares F test but not Tukey's least square means test.

Watering experiment

Mortality

Seedling mortality was pronounced in the two lowest moisture levels (5% and 10% CC), but no mortality occurred in any of the higher moisture level treatments. Mortality was significantly higher at 5% CC (55% mortality) than 10% CC (15% mortality; $p<0.0001$) and was higher without (46% mortality) than with field soil added (25% mortality; $P < 0.0001$).

Growth

Both watering and field soil addition significantly affected seedling height and shoot, root, and total biomass ($p<$ 0.0001 for all factors), with significant interactions between watering and soil for all variables ($p<0.0001$, Table 3). Seedlings grew larger with each increment in watering as well as with the addition of field soil, but the increases in shoot biomass leveled off at higher watering levels with field soil and were more linear without field soil (Fig. 2). Soil addition had the greatest proportional effects on seedling growth at 20–30% CC, but these increases diminished at field capacity. Although total biomass was greatest at field capacity, adding soil to 20–30% CC resulted in seedlings that were almost as large as those grown at field capacity without soil (Fig. 2).

Fungal colonization

Moisture affected all measures of fungal colonization except the proportion of root tips colonized by group 2 (*Wilcoxina*; Tables 2 and 4). Colonization by groups 1 and 3 were both highest at 30% CC (M10), which did not differ

Table 2 Proportional colonization of the three cluster groups of ectomycorrhizal morphotypes (see text for group compositions) where field soil was added

Treatment	Group 1	Group 2	Group 3	p value
Moisture				
M6 (100% CC)	$^{a}0.0000^{a}$ (0)	$^{a}0.0000^{a}$ (0)	$^{a}0.0264^{a}$ (0.0207)	0.0497
M8 (10% CC)	$^{b}0.1629^{a}$ (0.0256)	$^{a}0.0059^{b}$ (0.00353)	$^{b}0.0628^{c}$ (0.0108)	<0.0001
M10 (30% CC)	^{b}**0.1730**^{a} (0.0177)	^{a}**0.0074**^{b} (0.00810)	^{b}**0.0762**^{c} (0.0158)	<0.0001
p value	<0.0001	0.3184	0.0098	
Aeration				
A6	$^{a}0.5745^{a}$ (0.0597)	$^{ab}0.3784^{a}$ (0.0374)	$^{ab}0.0091^{b}$ (0.00429)	<0.0001
A7	$^{a}0.6205^{a}$ (0.0535)	$^{a}0.2626^{b}$ (0.0462)	^{a}**0.0771**^{c} (0.0253)	<0.0001
A8	$^{a}0.4619^{a}$ (0.0322)	^{b}**0.4803**^{a} (0.0374)	$^{ab}0.0175^{b}$ (0.00612)	<0.0001
A9	$^{a}0.5592^{a}$ (0.0442)	$^{ab}0.3823^{a}$ (0.0462)	$^{ab}0.0236^{b}$ (0.0104)	<0.0001
A10	^{a}**0.6408**^{a} (0.0445)	$^{ab}0.3201^{b}$ (0.0404)	$^{b}0.0000^{c}$ (0)	<0.0001
p value	0.1204	0.0152	0.0026	
Nitrogen				
N6	$^{a}0.0934^{a}$ (0.0261)	$^{a}0.3208^{b}$ (0.0572)	^{a}**0.0149**^{c} (0.00478)	<0.0001
N7	^{b}**0.4005**^{a} (0.0425)	$^{b}0.5295^{b}$ (0.0443)	$^{ab}0.0028^{c}$ (0.00180)	<0.0001
N8	$^{b}0.2460^{a}$ (0.0389)	$^{bc}0.6462^{b}$ (0.0351)	$^{ab}0.0085^{c}$ (0.00354)	<0.0001
N9	$^{b}0.2954^{a}$ (0.0360)	$^{bc}0.6663^{b}$ (0.0377)	$^{b}0.0000^{c}$ (0)	<0.0001
N10	$^{a}0.1117^{a}$ (0.0269)	^{c}**0.8026**^{b} (0.0329)	$^{ab}0.0082^{c}$ (0.00342)	<0.0001
p value	<0.0001	<0.0001	0.0184	
Phosphorus				
P6	$^{a}0.4646^{a}$ (0.0838)	^{a}**0.4983**^{a} (0.0820)	$^{a}0.0041^{b}$ (0.00289)	<0.0001
P7	$^{b}0.7394^{a}$ (0.0286)	$^{b}0.2023^{b}$ (0.0243)	$^{ab}0.0150^{c}$ (0.0105)	<0.0001
P8	$^{ab}0.5338^{a}$ (0.0526)	$^{b}0.2800^{b}$ (0.0525)	^{b}**0.0216**^{c} (0.00818)	<0.0001
P9	$^{b}0.7893^{a}$ (0.0562)	$^{b}0.1695^{b}$ (0.0491)	$^{ab}0.0059^{c}$ (0.00538)	<0.0001
P10	^{b}**0.8624**^{a} (0.0296)	$^{b}0.0942^{b}$ (0.0283)	$^{ab}0.0151^{b}$ (0.0152)	<0.0001
p value	<0.0001	<0.0001	0.0305	

Within a row, mean values followed by different letters indicated groups that were significantly different from each other at $p<0.05$. Within a column, mean values following different letters indicated treatments within each experiment that were significantly different from each other at $p<0.05$. For each group, the greatest colonization level within an experiment is in bold (standard error in parentheses)

Table 3 Comparison of seedling morphological parameters within watering and container media treatments for variables with significant interaction effects (standard error in parentheses)

Variable	Treatment	Moisture level										
		5% CC		10% CC		20% CC		30% CC		100% CC		*P*
Height (cm)	MB	5.21[a]	(0.333)	7.41[b]	(0.157)	9.27[c]	(0.203)	12.1[d]	(0.276)	23.8[f]	(0.540)	<0.0001
	MS	5.86[ab]	(0.357)	9.04[bc]	(0.199)	12.8[d]	(0.328)	14.9[e]	(0.379)	25.4[f]	(0.600)	
	p	1.00		0.0708		<0.0001		<0.0001		0.0878		
Total biomass (g)	MB	0.256[a]	(0.0171)	0.385[a]	(0.0111)	0.784[bd]	(0.0211)	0.995[d]	(0.0255)	2.30[e]	(0.0760)	<0.0001
	MS	0.315[a]	(0.0204)	0.689[b]	(0.0172)	1.70[c]	(0.0614)	1.90[c]	(0.0622)	2.66[f]	(0.0981)	
	p	1.00		0.0010		<0.0001		<0.0001		<0.0001		
R/S	MB	0.534[a]	(0.0224)	0.545[a]	(0.0184)	0.701[b]	(0.0191)	0.554[a]	(0.0144)	0.338[c]	(0.0211)	<0.0001
	MS	0.505[a]	(0.0253)	0.595[ab]	(0.0239)	0.345[c]	(0.0225)	0.438[ac]	(0.0246)	0.459[a]	(0.0412)	
	p	1.00		1.00		<0.0001		0.0265		0.0169		

Treatment means not sharing the same superscript across rows are different at $p \leq 0.05$. Exact *p* values for container media contrasts within a watering treatment level are presented below the means for that variable

MB basic container media treatment, *MS* basic container media + soil treatment

significantly from 10% CC (M8; $p>0.05$). Consistent with this, total colonization and all measures of taxa diversity (richness and Simpson's index) were highest in the lower moisture levels, M8 and M10.

Aeration experiment

Growth

Mortality was negligible across Styrolite® treatments, but growth was significantly affected. Seedling diameter decreased with increasing Styrolite® volume ($p=0.0040$), but was unaffected by addition of field soil ($p=0.9486$), and there was no interaction between Styrolite® and soil addition ($p=0.2159$, Table 5). Seedling height varied with both Styrolite® ($p=0.0234$) and field soil treatments ($p<0.0001$), and the interaction between the two main effects was significant ($p<0.0001$, Table 6). Seedling height was greater in the basic medium than when field soil was added, but the difference between field soil treatments decreased with increasing Styrolite® content. Seedling height decreased with increasing Styrolite® in the basic medium, but was generally unaffected by Styrolite® where field soil was added.

Styrolite® addition and field soil interacted significantly in their effects on shoot, root, and total biomass ($p<0.0001$, Table 6 and Fig. 3). Where no field soil was added to the growing medium, shoot, root, and total biomass generally declined steadily with increasing Styrolite®, with optimal conditions for biomass growth consistently at 100% peat. Addition of field soil, however, eliminated growth losses at higher Styrolite® levels. Both root biomass and R/S were generally higher when field soil was added, particularly at 10% Styrolite®. Consistent with total biomass, adding 40% Styrolite® was suppressive to seedling root growth regardless of field soil addition.

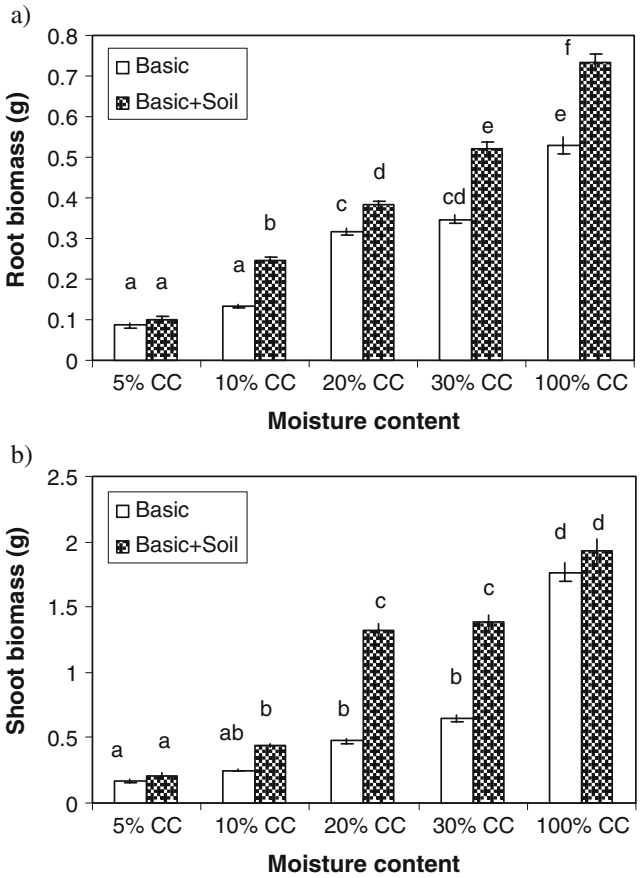

Fig. 2 Comparison of seedling root biomass (**a**) and shoot biomass (**b**) among watering and field soil treatments. Both water and soil treatment effects interacted significantly ($p<0.0001$). "Basic" refers to the basic growing medium (0.5 m^3 Rich Grow Peat® moss, 0.5 m^3 Sun Grow Peat® moss, 1.0 kg dolomite, 1.0 kg gypsum, 0.1 m^3 Styrolite®), and "Basic plus Soil" refers to the basic growing medium plus 0.1 m^3 field soil

Table 4 Comparison of fungal community parameters among moisture levels for the basic container media + soil treatment (standard error in parentheses)

Variable	Moisture level						
	10% CC		30% CC		100% CC		*P*
Percent colonization	0.231[a]	(0.0265)	0.244[a]	(0.0284)	0.0333[b]	(0.0207)	<0.0001
Richness	2.33[a]	(0.142)	2.08[a]	(0.149)	0.250[b]	(0.131)	<0.0001
Simpson's index	0.653[a]	(0.0502)	0.598[a]	(0.0467)	0.250[b]	(0.131)	0.0043

Moisture treatment means not sharing the same superscript are different at $p \leq 0.05$

Fungal colonization

Simpson's diversity index and total colonization were not affected by aeration ($p \geq 0.05$) (Tables 2 and 7). Richness was highest under the lower Styrolite® levels. Percent colonization by group 1 did not respond significantly to Styrolite®. Group 2 increased with increasing Styrolite®, while group 3 had the reverse pattern (Table 2).

Nitrogen experiment

Growth

Mortality was negligible throughout the N experiment. Both N and field soil addition significantly affected all growth parameters, and there were significant interactions between N and soil for diameter, root biomass, total biomass, and R/S (Table 8). Seedling diameter, height (Table 9), shoot biomass, and total biomass increased with increasing N addition, with the greatest growth at the highest N level ($p<0.0001$). Addition of field soil to the growing medium generally resulted in slower height growth ($p=0.0116$), lower shoot biomass ($p=0.0044$), and lower total biomass ($p<0.0001$), except at the highest N level where adding field soil did not have a suppressive effect. In contrast, diameter growth was generally improved by field soil addition ($p=0.0063$).

For the basic soil treatment, root biomass was significantly reduced by the highest N level but did not vary among the four lowest N rates ($p<0.0001$, Fig. 4). With the addition of field soil, root biomass was significantly greater at N20 and N40 than any of the other N treatments ($P < 0.0001$). Root to shoot ratio declined steadily with increasing N addition and was highest at N10 followed by N20 and N40 ($p<0.0001$, Fig. 4). Addition of field soil caused R/S to decline in N10 and N100 relative to the basic medium.

Fungal colonization

Nitrogen addition affected ($p=0.05$) all measures of fungal colonization (Tables 2 and 10). Richness and Simpson's index were erratic in their response to N. Total colonization and colonization by group 2 were lowest at low N (Table 2) while that of groups 1 and 3 were generally highest under low levels of N.

Phosphorus experiment

Growth

Mortality was negligible throughout the P experiment. Phosphorus addition significantly affected all growth parameters except diameter, and soil addition affected all parameters, including increased R/S under the highest level of P (Table 11 and Fig. 5). There were no significant interactions between P and field soil addition for any parameter other than root biomass and R/S. Seedling height, shoot biomass, and total biomass were significantly higher at P25 and lower at P10 compared to all of the other P treatments ($p<0.0001$ for all variables). Shoot responses did not differ among P5, P15, or P60. The effects of P on root biomass were opposite of those on shoots. Differences

Table 5 Comparison of seedling morphological parameters among Styrolite® and container media treatments for variables without significant interaction effects (standard error in parentheses)

Styrolite® level											Container media				
Variable	Peat only		Peat+10%		Peat+20%		Peat+30%		Peat+40%	*p*	Basic		Basic+soil		*p*
Diameter (mm)	4.03[a]	(0.188)	3.73[ab]	(0.0482)	3.78[ab]	(0.0465)	3.63[b]	(0.0486)	3.55[b]	(0.0405)	0.004	3.74	(0.0783)	3.75	(0.0313)	0.949

Styrolite treatment means not sharing the same superscript are different at $p \leq 0.05$

Table 6 Comparison of seedling morphological parameters within Styrolite® and container media treatments for variables with significant interaction effects (standard error in parentheses)

Variable	Treatment		Styrolite® level										
			Peat only		Peat+10%		Peat+20%		Peat+30%		Peat+40%		*p*
Height (cm)	MB[1]		36.4[a]	(0.591)	35.5[a]	(0.607)	34.6[ac]	(0.618)	33.0[c]	(0.481)	31.2[cd]	(0.420)	<0.0001
	MS		26.8[b]	(0.522)	28.4[bd]	(0.445)	28.9[bd]	(0.494)	28.6[bd]	(0.567)	29.8[d]	(0.525)	
		p	<0.0001		<0.0001		<0.0001		<0.0001		1.00		
Shoot biomass (g)	MB		3.09[a]	(0.0906)	2.83[ab]	(0.0881)	2.81[ab]	(0.0779)	2.59[b]	(0.0838)	2.39[b]	(0.0676)	<0.0001
	MS		3.04[a]	(0.0695)	2.64[b]	(0.0814)	2.99[ab]	(0.0711)	3.21[a]	(0.0652)	3.24[a]	(0.0747)	
		p	1.00		1.00		1.00		<0.0001		<0.0001		
Root biomass (g)	MB		0.974[a]	(0.0293)	0.941[ac]	(0.0287)	0.889[ac]	(0.0263)	0.816[c]	(0.0269)	0.854[ac]	(0.0208)	0.0016
	MS		1.02[ab]	(0.0395)	1.12[b]	(0.0365)	1.02[ab]	(0.0321)	1.11[ab]	(0.0365)	0.972[a]	(0.0291)	
		p	1.00		0.0018		0.157		<.0001		0.332		

Treatment means not sharing the same superscript across rows are different at $p \leq 0.05$. *P* values for container media contrasts within a Styrolite® treatment level are presented below the means for that variable

MB basic container media treatment, *MS* basic container media+soil treatment

in shoot and root responses are reflected in R/S (Fig. 5). As with root biomass, R/S was highest in P10, lowest in P25, and intermediate in P5, P25, and P50. Addition of field soil had a weakly suppressive effect on seedling height, shoot biomass, and total biomass across all P levels except at P10 where there was no effect. Similarly, addition of field soil resulted in slightly lower root biomass in all treatments except P60 (Fig. 5).

Fungal colonization

Phosphorus affected percent fungal colonization, but not measures of ECM fungal diversity (Table 12). Percent colonization by group 1 increased with P (Table 2). Conversely, group 2 decreased with P (Table 2). Colonization by group 3 peaked in P3 (Table 2). Total colonization was minimized in P3.

Discussion

ECM fungi and functional niches

ECM fungi have functional niches often corresponding to succession on individual plant roots as well as at the community level (Mason et al. 1983; Deacon and Fleming 1992; Newton 1992; Johnson et al. 2006; Twieg et al. 2007). Succession has been thought as driven primarily by changes in soil nutrient availability, size of the carbon (C) source, the rate at which various fungal species colonize, and the presence and proximity of plant and fungal species. In our study, most fungal morphotypes were clustered into the group (group 3) that field studies have shown are

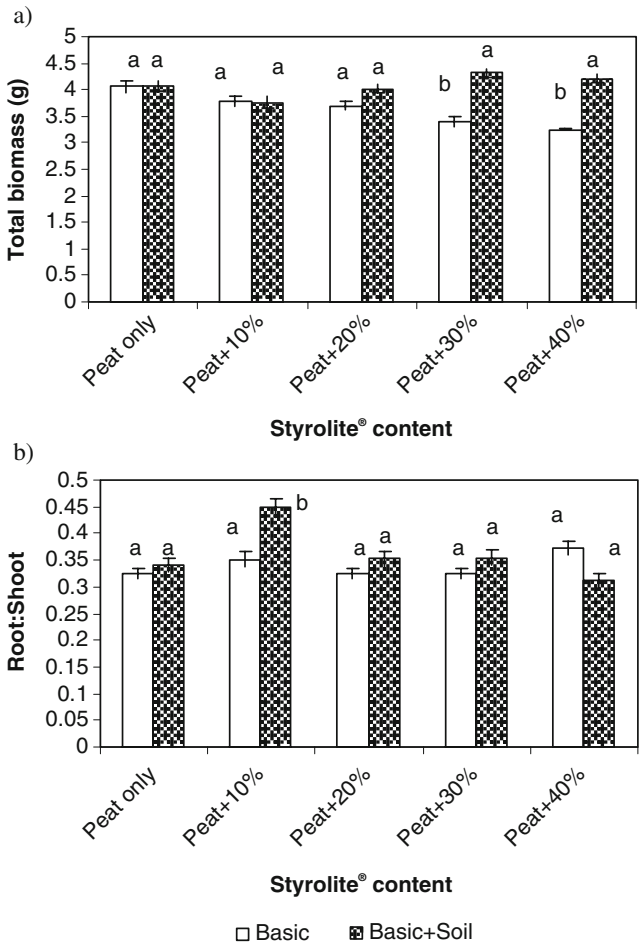

Fig. 3 Comparison of total biomass (**a**) and root/shoot (**b**) among aeration and field soil treatments. There were significant interactions between the main effects for both variables ($p<0.0001$)

Table 7 Comparison of fungal community parameters among Styrolite® levels for the basic container media+soil treatment (standard error in parentheses)

Variable	Styrolite® level										
	Peat only		Peat+10%		Peat+20%		Peat+30%		Peat+40%		*p*
Percent colonization	0.963[a]	(0.00543)	0.951[a]	(0.0121)	0.954[a]	(0.0273)	0.961[a]	(0.00640)	0.953[a]	(0.0140)	0.818
Richness	2.75[ab]	(0.218)	3.00[b]	(0.275)	3.00[b]	(0.174)	2.50[ab]	(0.195)	2.00[a]	(0)	0.0032
Simpson's index	0.600[a]	(0.0368)	0.571[a]	(0.0378)	0.523[a]	(0.0285)	0.560[a]	(0.0238)	0.573[a]	(0.0217)	0.5036

Styrolite® treatment means not sharing the same superscript are different at $p \leq 0.05$

abundant in more advanced stages of seedling establishment in Douglas-fir forests (Twieg et al. 2007; Teste et al. 2009). This group was abundant and co-dominant at 30% soil water field capacity, low N levels, and intermediate P levels, which emulates advanced forest conditions and suggests that these fungi are limited under conditions characteristic of recently disturbed sites and container nursery conditions (i.e., high N/P ratios and ample water). The decline in group 3 with increasing aeration (and Styrolite®) may have been a direct response to the decline in organic matter content of the substrate, which is depleted with disturbance and increases with succession (Chapin et al. 2002). Alternatively, water and/or P levels may have declined enough with Styrolite® addition to directly limit the fungi. However, there was also an abrupt decrease in colonization by group 3 from 10% Styrolite® to 100% peat moss, where aeration was low enough and nutrient availability or organic matter content high enough to suppress colonization by this functional group.

Group 2, consisting exclusively of *Wilcoxina*, behaved as would be expected for a rapidly establishing, early-successional colonizer that thrives under high nutrient conditions with lower C requirements. Group 2 was dominant at high N/P ratios and 20% Styrolite®, one level higher than that of group 3, suggesting that both high nutrient availability and low organic content favored this group. Lowered nutrient availability at higher levels of Styrolite® addition may have limited colonization by group 2. The major limitation of this successional analogy is the short time frame of the experiments, the exclusive use of seedlings, and the highly artificial conditions. Effects of tree age, C supply, and plant physiology on fungal community composition were not captured here, and *Wilcoxina rehmii* not only can dominate early seres but is also multi-successional. *Thelephora terrestris* (clustered in group 3) has also been found to occur in early-successional stages in mesic Douglas-fir forests (Chapman 1991; Twieg et al. 2007; Teste et al. 2009); thus, its association with group 3 in this study suggests that its waning colonization with succession may be related to antagonism with more K-selected fungi.

Group 1 (consisting of DSE) appears to be facilitated by earlier successional conditions than group 3, with higher abundance under higher levels of N and P than group 3. Dark septate endophytes have been found to coexist with other ECM fungi during Douglas-fir forest establishment (Horton et al. 1998; Twieg et al. 2007; Teste et al. 2009), possibly serving as pioneers during succession (Horton et al. 1998, Cázares et al. 2005). Little is known about the function of DSE (Mandyam and Jumpponen 2005), but

Table 8 Comparison of seedling morphological parameters within N and container media treatments for those variables having significant interaction effects (standard error in parentheses)

Variable	Treatment	Nitrogen level										
		N1		N2		N3		N4		N5		*p*
Diameter (mm)	MB[1]	2.48[a]	(0.0384)	3.02[b]	(0.0433)	3.19[bc]	(0.0513)	3.40[c]	(0.0559)	3.35[c]	(0.0601)	0.0144
	MS	2.63[a]	(0.0417)	3.16[bc]	(0.0527)	3.31[c]	(0.0548)	3.27[c]	(0.0483)	3.54[c]	(0.0588)	
	p	1.00		1.00		1.00		0.401		1.00		
Total biomass (g)	MB	1.83[a]	(0.0445)	2.69[b]	(0.0602)	3.19[c]	(0.0912)	3.14[c]	(0.0840)	3.26[c]	(0.0966)	0.0466
	MS	1.64[a]	(0.0469)	2.47[b]	(0.0641)	2.87[bc]	(0.0765)	2.81[b]	(0.0588)	3.32[c]	(0.0834)	
	p	1.00		1.00		0.0927		0.0482		1.00		

Treatment means not sharing the same superscript across rows are different at $p \leq 0.05$. *P* values for container media contrasts within an N treatment level are presented below the means for that variable

MB basic container media treatment, *MS* basic container media+soil treatment

Table 9 Comparison of seedling morphological parameters among N and container media treatments for those variables without interaction effects (standard error in parentheses)

Variable	Nitrogen level										Container media				
	N1		N2		N3		N4		N5	*p*	Basic		Basic+soil		*p*
Height (cm)	19.4[a]	(0.268)	30.1[b]	(0.381)	33.3[c]	(0.399)	31.1[b]	(0.398)	36.2[d]	(0.530)	<0.0001	30.5	(0.403)	29.6	(0.386)	0.0116
Shoot biomass (g)	0.983[a]	(0.0239)	1.79[b]	(0.0359)	2.26[c]	(0.0514)	2.24[c]	(0.0436)	2.62[d]	(0.0573)	<0.0001	2.04	(0.0418)	1.92	(0.0394)	0.0044

Nitrogen treatment means not sharing the same superscript are different at $p \leq 0.05$

they produce extraradical hyphae and thus increase the potential surface area for the uptake of soil nutrients (Jumpponen 2001). The increase in colonization with moisture in the watering experiment may, at first glance, appear to contradict the results of Addy et al. (2000) who seemingly found no habitat specificity along a moisture gradient of the DSE *Phialocephala fortinii*; however, they only sampled for the presence of this endophyte, not levels of colonization. That we found DSE responding to N levels disagrees with Mandyam and Jumpponen (2008), but their study differed because the tallgrass prairie plants and community were already well established, the experiment occurred over a longer time frame, and the community was less N-limited than the late-successional Douglas-fir forests that our fungi evolved in. We also found DSE abundant at high N/P, suggesting that DSE may be multi-successional on Douglas-fir. The different conditions facilitating group 1 and group 3 may also have resulted from antagonism.

Effects of resource limitations on ECM importance to plants

Water

In the watering experiment, adding field soil to the growing medium reduced mortality at the two lowest moisture contents. This was likely due to improved water uptake resulting from greater mycorrhization. Field soil addition at field capacity increased total biomass, but most of this was allocated to the roots, and mortality was not reduced. This suggests that mycorrhization incurred a benefit to surviving seedlings in wet conditions via increased biomass, but most of this increased biomass was allocated to the roots, thus potentially incurring the greatest competitive advantage when a soil nutrient is limiting. Our results corroborate those of Parke et al. (1983) who found that both shoot and root biomass of Douglas-fir seedlings were maximized when the seedlings were ectomycorrhizal and kept at field capacity and minimized when they were non-mycorrhizal and subjected to cyclical drought. In their experiment, the highest R/S ratio occurred in the non-mycorrhizal drought treatment while the lowest R/S occurred in the mycorrhizal drought treatment. They reported no mortality for these low water treatments, making it likely that the level of water stress was similar to our 20% CC treatment where we too observed the most extreme responses of R/S to ectomycorrhizal colonization. Furthermore, Runion et al. (1994) found higher

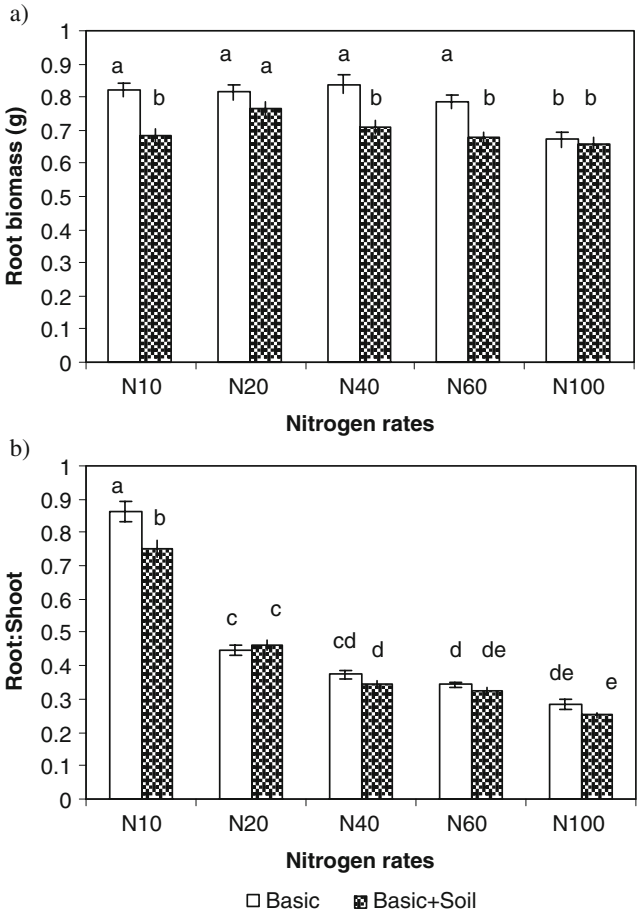

Fig. 4 Comparison of root biomass (**a**) and root/shoot (**b**) among N and field soil treatments. Nitrogen and soil interactions were significant for both variables ($p<0.05$)

Table 10 Comparison of fungal community parameters among N levels for the basic container media+soil treatment (standard error in parentheses)

Variable	Nitrogen level										
	N1		N2		N3		N4		N5		*p*
Percent colonization	0.421[a]	(0.0607)	0.930[b]	(0.0157)	0.895[b]	(0.0185)	0.957[b]	(0.00892)	0.900[b]	(0.0194)	<0.0001
Richness	2.33[ab]	(0.188)	2.25[ab]	(0.131)	2.67[b]	(0.188)	2.00[a]	(0)	2.25[ab]	(0.131)	0.0382
Simpson's index	0.630[ab]	(0.0547)	0.608[b]	(0.0431)	0.658[ab]	(0.0466)	0.578[b]	(0.0286)	0.794[a]	(0.0428)	0.0114

Nitrogen treatment means not sharing the same superscript are different at $p \leq 0.05$

growth and ectomycorrhizal colonization of longleaf pine (*Pinus pallustris* Mill) seedlings grown with adequate water relative to those grown under drought stress. Ectomycorrhizas have been shown to increase resistance of trees to drought-induced water stress by increasing water uptake and increasing physiological regulation of water use (Garbaye 2000), with some ectomycorrhizal fungi more beneficial to trees under stress than other fungi (Jany et al. 2003).

Seedlings without mycorrhizas are likely able to access similar amounts of soil water as seedlings with mycorrhizas when all of the soil pores are filled (i.e., the benefits of mycorrhization are lost because water is readily available to non-mycorrhizal roots); thus, mycorrhization does not incur strong advantages for water uptake at high water levels. In the watering experiment, mortality ranged from nearly zero to almost 70%, indicating that our treatments represented a broad range of water stress relative to this population's adaptive envelope; thus, we believe that our intermediate moisture levels correspond roughly to the intermediate levels of the population's realized niche. The greatest disparity in growth between high colonization (with field soil) and low colonization (without field soil) seedlings in our study was at these intermediate moisture levels, which complements the findings of Swaty et al. (2004) that ECM colonization was most enhanced at intermediate water availability relative to the realized niche of the conifer host (*Pinus edulis*). At the lowest moisture levels in our study, ECM mycelium growth was likely limited to the degree that it was ineffective at increasing conifer growth (Jany et al. 2003; Duñabeitia et al. 2004). At the highest moisture levels, in contrast, conifers were likely acquiring sufficient moisture with their own root system such that mycorrhizas did not incur a benefit. This cost/benefit trade-off is predicted by both the economic model of mutualism (Schwartz and Hoeksema 1998; Hoeksema and Bruna 2000; Hoeksema and Kummel 2003; Hoeksema and Schwartz 2003; Johnson et al. 2006) and the functional equilibrium model (Brouwer 1983; Treseder and Allen 2002; Johnson et al. 2003, 2006). The disparity in total biomass between high colonization (with field soil) and low colonization (without field soil) seedlings at intermediate moisture levels was almost completely due to differences in shoot biomass in our study, further supporting an increased reliance on ECM fungi instead of roots for water uptake.

Aeration

The aeration experiment showed that root development was maximized by a 10% addition of Styrolite®, provided field soil was added. A plausible reason for this is that higher nutrient content in the lowest Styrolite® level (due to greater water retention and cation exchange capacity) may have reduced biomass allocation to roots, whereas low nutrients at the highest Styrolite® levels may have resulted

Table 11 Comparison of seedling morphological parameters among P and container media treatments for those variables without interaction effects (standard error in parentheses)

Variable	Phosphorus level										Container media				
	P1		P2		P3		P4		P5	*p*	Basic		Basic+soil		*p*
Height (cm)	33.6[a]	(0.425)	30.1[b]	(0.405)	34.8[ac]	(0.420)	36.3[c]	(0.522)	34.3[a]	(0.542)	<0.0001	34.6	(0.302)	33.4	(0.311)	0.0057
Diameter (mm)	3.68[a]	(0.0472)	3.59[a]	(0.0447)	3.64[a]	(0.0481)	3.69[a]	(0.0482)	3.74[a]	(0.0486)	0.246	3.71	(0.0309)	3.62	(0.0288)	0.0429
Shoot biomass (g)	2.49[a]	(0.0594)	2.10[b]	(0.0425)	2.55[a]	(0.0603)	2.86[c]	(0.0611)	2.51[a]	(0.0647)	<0.0001	2.58	(0.0396)	2.43	(0.0377)	0.0034
Total biomass (g)	3.51[a]	(0.0703)	3.22[b]	(0.0571)	3.58[a]	(0.0785)	3.77[a]	(0.0694)	3.47[ab]	(0.0783)	<0.0001	3.61	(0.0466)	3.41	(0.0445)	0.0014

Phosphorus treatment means not sharing the same superscript are different at $p \leq 0.05$

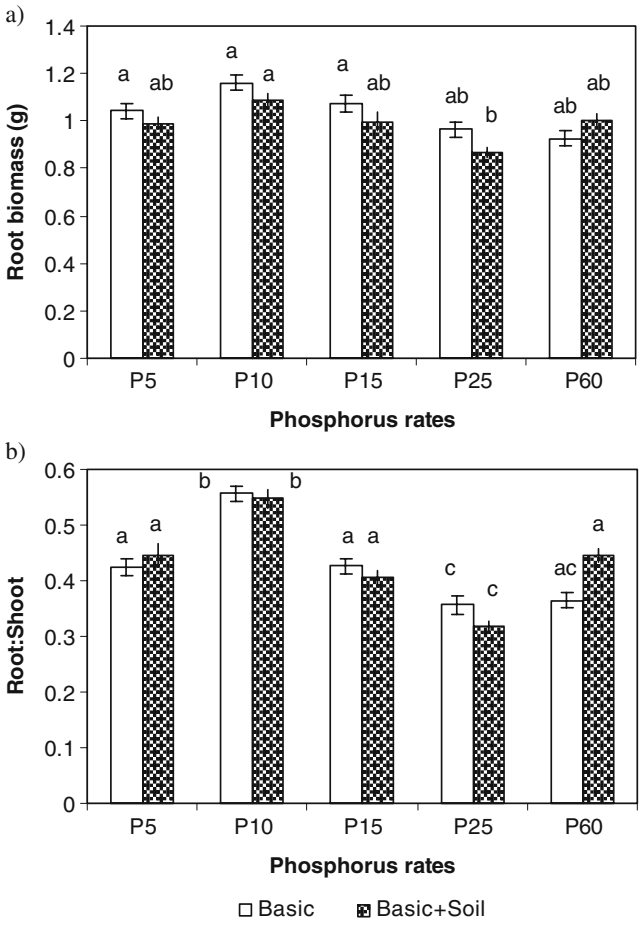

Fig. 5 Comparison of root biomass (**a**) and root/shoot (**b**) among P and soil inoculation treatments. There were significant interactions between the main effects for both variables ($p<0.05$)

in more allocation to mycorrhizal fungi at the expense of root growth. Increasing Styrolite® reduced seedling diameter and height growth in the absence of field soil, but field soil addition equalized the height among Styrolite® treatments. Shoot and total biomass also decreased with increasing Styrolite®, unless field soil was added, which reversed the trend. The reduced growth with displacement of peat by Styrolite® in the absence of field soil may have resulted from either reduced soil water availability (as indicated by the decline in water holding porosity) or reduced soil nutrient availability. Rincón et al. (2005) found that ECM inoculation had the greatest effect on *Pinus pinea* seedling foliar nutrient content when seedlings were grown in a substrate with no mineral component. Whereas our 100% organic treatment comprised peat, that of Rincon et al. consisted of 50% composted acacia bark with a high pH, which may have facilitated nutrient uptake by the mycorrhizal fungi. Unlike Rincón et al. (2005) who found no interaction between substrate and mycorrhizal colonization in their effects on growth, we found that adding field soil overcame the imposed deficiency created by adding Styrolite®. Not only did adding field soil overcome this deficiency but also total biomass was greatest under the two highest levels of Styrolite® when field soil was added despite total biomass being lowest at these same levels when no field soil was added. Where field soil was added, shoot and total biomass increased with Styrolite® addition, suggesting that aeration was beneficial to seedling biomass growth provided the imposed reduction in nutrient availability with peat displacement was compensated by field soil addition. Added field soil may have improved nutrient availability to seedlings by increasing the available nutrient content in soil solution, by increasing the cation exchange capacity, and/or by increasing nutrient uptake through mycorrhizal inoculation.

Nitrogen

In Douglas-fir ecosystems, N is the most common limiting soil nutrient and Douglas-fir seedling establishment is most successful under conditions of high N (Jurgensen et al. 1997). High levels of N fertilization improve Douglas-fir shoot growth, which help it to compete for light when planted in plant communities with rapid height growth, but can reduce root growth, reducing competitiveness in communities that are competitive belowground for soil resources. Reductions in shoot growth resulting from addition of field soil in our experiment were likely due to greater colonization by mycorrhizal fungi. A number of studies have examined the interaction between N and

Table 12 Comparison of fungal community parameters among P levels for the basic container media + soil treatment (standard error in parentheses)

Variable	Phosphorus level										
	P1		P2		P3		P4		P5		*p*
Percent colonization	0.961[a]	(0.0114)	0.967[a]	(0.00651)	0.840[b]	(0.0267)	0.962[a]	(0.00840)	0.980[a]	(0.00705)	0.0004
Richness	2.58[a]	(0.229)	2.83[a]	(0.271)	3.25[a]	(0.179)	2.67[a]	(0.142)	3.17[a]	(0.207)	0.1095
Simpson's index	0.647[a]	(0.0555)	0.595[a]	(0.0236)	0.641[a]	(0.0490)	0.688[a]	(0.0427)	0.783[a]	(0.0414)	0.3853

Phosphorus treatment means not sharing the same superscript are different at $p\leq0.05$

mycorrhizal colonization (Reid et al. 1983; Hobbie and Colpaert 2003). While Reid et al. (1983) found that biomass of pine (*Pinus taeda*) increased with ECM colonization under N fertilization, Hobbie and Colpaert (2003) found that biomass of *Pinus sylvestris* was reduced with ectomycorrhizal colonization under N enrichment, as in our study. We found that field soil addition decreased aboveground biomass the most at intermediate N fertilization rates, while Hobbie and Colpaert (2003) found this to occur at the lowest N enrichment treatment.

Our results indicate that while field soil addition suppressed root growth at lower levels of N in Douglas-fir as we expected, it reduced shoot growth across these same levels of N, which we did not expect. Addition of field soil reduced R/S only at N10, not the higher N treatments, suggesting that seedlings benefited more by investing in mycorrhizal mycelia than root growth. In the intermediate treatments (N20 to N60), increased mycorrhizal colonization with field soil addition consistently reduced total biomass, root biomass, shoot biomass, and height. At N100, in contrast, field soil addition did not reduce growth or increase biomass allocation to roots, likely because the plant could easily attain N at sufficient rates without the aid of fungi, and thus, the most efficient allocation of C would be primarily to its own biomass.

Phosphorus

While N is most often limiting in Douglas-fir forests, P can be limiting as well, such as when the primary inputs are from weathering of recently deposited glacial till. Despite P limitations in some Douglas-fir forests, our results are consistent with the possibility that water and N limitations, which predominate throughout the range of Douglas-fir, have selected for Douglas-fir mutualists that are poor at P uptake. These mycorrhizal fungi are probably parasitizing seedlings when water and N are sufficient, as we found that field soil addition reduced total biomass under every P treatment. We also found that field soil addition enhanced root growth, increased R/S, and reduced shoot biomass at P60 relative to P25. This suggests that the highest level of P fertilization induced N limitation (Conjeaud et al. 2006). Supporting this, the lowest R/S occurred at P25, suggesting an ideal P/N ratio for complete utilization of the fertilizers, while the greatest R/S occurred at P10, suggesting that P was limiting.

A number of studies have looked at interactions between soil P and ectomycorrhizal colonization of trees (Twieg et al. 2009). In forests similar to the one where we collected soils, Twieg et al. (2009) found that the relationships between measured soil variables and ECM communities were too complex to reliably model with the available data. In our greenhouse experiment, the potential benefits of increased mycorrhization to C capture (i.e., increased assimilation rates) with P addition were outweighed by their C cost to the seedling, possibly because the nursery mycorrhizas were poor at P uptake, the added P induced N limitations, and/or because soil P was more readily available in the small containers. As with our study, Conjeaud et al. (2006) found that P enrichment and ECM inoculation of nursery-grown Maritime pine (*Pinus pinaster* Soland. *in* Ait.) reduced seedling biomass, even though it increased both net photosynthesis and root respiration rates.

Conclusions

Both the presence of inoculum (field soil) and the availability of soil nutrients are factors affecting mycorrhizal fungal colonization and community structure. We found that root development of interior Douglas-fir seedlings can be increased with adjustments to the water, aeration, N, and P regimes currently used in commercial nurseries and that adjustments will also affect the mycorrhizal community in a way that reflects the successional conditions to which the fungi are adapted. Further research is needed to test whether the long-term success of Douglas-fir regeneration can be improved by adjusting cultivation factors to maximize R/S or favor ECM fungal communities that correspond to later seral stages, rather than maximizing total or shoot biomass, and whether this varies depending on the planting site and species. We also found that adding field soil to the growing medium substantially increased mycorrhization and had a large effect on Douglas-fir seedling growth, which may have occurred through mycorrhization, nutrient addition, or effects on water holding capacity. Accordingly, in most experiments, field soil addition had the least effect when nutrient conditions were most ideal for whole seedling growth.

The N and P fertilization rates that maximized R/S and colonization by group 3 (more advanced successional fungi) are lower than that recommended by Hunt (1992) for mycorrhization of container-grown Engelmann spruce and lodgepole pine, but these species form mycorrhizas more readily than interior Douglas-fir. Our results suggest that by reducing fertilizer and water applications, nurseries may be able to reduce costs while still retaining high establishment success due to increased mycorrhization and root growth. Douglas-fir does not generally have high fine root density in nursery plugs, but rather coarse roots that are not colonized with beneficial fungi. Seedling establishment success rates should increase with mycorrhization when the fungi are aiding in access to a limiting nutrient or are antagonizing parasitic fungi. Adding field soil to the growing substrate should facilitate mycorrhization in greenhouse environments. Future studies on seedling

growth and mycorrhizal responses to cultivation conditions should test the interactive effects of all factors, different proportions of field soil addition, and multiple tree species. These seedlings should then be tested along environmental gradients in the field to determine long-term establishment success and how it relates to mycorrhization in nursery conditions.

References

Addy HD, Hambleton S, Currah RS (2000) Distribution and molecular characterization of the root endophyte *Phialocephala fortinii* along an environmental gradient in the boreal forest of Alberta. Mycol Res 104:1213–1221

Agerer R (1987–1998) Color atlas of ectomycorrhizas. Einhorn-Verlag Eduard Dietenberger, Schwäbisch Gmünd

Berch SM, Xiao G, Bulmer C (1999) Commercial mycorrhizal inoculants: value added conifers for site rehabilitation or just another way to spend money? Proceedings of the 19th Annual Meeting of the Forest Nursery Association of British Columbia. Extension Services, Tree Improvement Branch, Surrey, Canada

Brouwer R (1983) Functional equilibrium: sense or nonsense? Netherlands J Agric Sci 31:335–348

Campbell DB, Jones MD, Kiiskila S, Bulmer C (2003) Two-year field performance of lodgepole pine seedlings: effects of container type, mycorrhizal fungal inoculants, and site preparation. BC J Ecosyst Manage 3:1–11

Cázares E, Trappe JM, Jumpponen A (2005) Mycorrhiza–plant colonization patterns on a subalpine glacier forefront as a model system of primary succession. Mycorrhiza 15:405–416

Chapin FS III, Matson PA, Mooney HA (2002) Principles of terrestrial ecosystem ecology. Springer, Berlin

Chapman WK (1991) Inoculation of ectomycorrhizal fungi in the IDFdk2 biogeoclimatic zone of British Columbia: new techniques, fungi and outplanting trials. PhD thesis, University of British Columbia, Vancouver, Canada

Conjeaud C, Scheromm P, Mousain D (2006) Effects of phosphorus and ectomycorrhiza on maritime pine seedlings (*Pinus pinaster*). New Phytol 133:345–351

Deacon JW, Fleming LV (1992) Interactions of ectomycorrhizal fungi. In: Allen MF (ed) Mycorrhizal Functioning: an integrative plant–fungal process. Chapman and Hall, London, pp 249–300

Dickie IA, Reich PB (2005) Ectomycorrhizal fungal communities at forest edges. J Ecol 93:244–255

Dickie IA, Koide RT, Steiner KC (2002) Influences of established trees on mycorrhizas, nutrition, and growth of *Quercus rubra* seedlings. Ecol Monogr 72:505–521

Dickie IA, Schnitzer SA, Reich PB, Hobbie SE (2005) Spatially disjunct effects of co-occurring competition and facilitation. Ecol Lett 8:1191–1200

Duñabeitia MK, Hormilla S, Garcia-Plazaola JI, Txarterina K, Arteche U, Becerril JM (2004) Differential responses of three fungal species to environmental factors and their role in the mycorrhization of *Pinus radiata* D Don. Mycorrhiza 14:11–18

Garbaye J (2000) The role of ectomycorrhizal symbiosis in the resistance of forests to water stress. Outlook Agric 29:63

Goodman DM, Durall DM, Trofymow JA, Berch SM (1996) Describing ectomycorrhizas: a manual of concise descriptions of North American ectomycorrhizas. Mycologue, Sydney

Greisbauer H (2008) Regional, ecological and temporal patterns in Douglas-fir climate–growth relationships in the British Columbia Interior. Master's thesis, University of Northern British Columbia, Prince George, Canada

Hartnett DC, Wilson GWT (2002) The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. Ecology 80:1187–1195

Heineman JL, Hope GD, Simard SW, Vyse A, Lloyd DL, Miege DJ (2003) The effects of site preparation and harvesting practices on planted seedling productivity and microenvironment in southern interior dry, grassy Interior Douglas-fir forests. British Columbia Ministry of Forests, Victoria, Canada. Tech. Rep. 009

Hobbie EA, Colpaert JV (2003) Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. New Phytol 157:115–126

Hoeksema JD, Bruna EM (2000) Pursuing the big questions about interspecific mutualism: a review of theoretical approaches. Oecologia 125:321–330

Hoeksema JD, Kummel M (2003) Ecological persistence of the plant–mycorrhizal mutualism: a hypothesis from species coexistence theory. Am Nat 162:S40–S50

Hoeksema JD, Schwartz MW (2003) Expanding comparative-advantage biological market models: contingency of mutualism on partners' resource requirements and acquisition trade-offs. Proc R Soc Lond Series B 270:913–919

Horton TR, Cazaras E, Bruns TD (1998) Ectomycorrhizal, vesicular–arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire. Mycorrhiza 7:11–18

Horton TR, Bruns TD, Parker VT (1999) Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. Can J Bot 77:93–102

Hunt GA (1992) Effects of mycorrhizal fungi on quality of nursery stock and plantation performance in the southern interior of British Columbia. FRDA Report ISSN 0835-0752; 185

Ingleby K, Mason PA, Last FT, Fleming LV (1990) Identification of ectomycorrhizas. HMSO London. ITE research publication no. 5

Jany J-L, Martin F, Garbaye J (2003) Respiration activity of ectomycorrhizas from *Cenococcum geophilum* and *Lactarius* sp. in relation to soil water potential in five beech forests. Plant Soil 255:487–494

Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism–parasitism continuum. New Phytol 135:575–586

Johnson NC, Rowland DL, Corkidi L, Egerton-Warburton L, Allen EB (2003) Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. Ecology 84:1895–1908

Johnson NC, Hoeksema JD, Bever JD, Chaudhary VB, Gehring C, Klironomos J, Koide R, Miller RM, Moore J, Moutoglis P, Schwartz M, Simard S, Swenson M, Umbanhowar J, Wilson G, Zabinski C (2006) From Lilliput to Brobdingnag: extending models of mycorrhizal function across scales. Bioscience 56:889–900

Jones MD, Smith SE (2004) Exploring functional definitions of mycorrhizas: are mycorrhizas always mutualisms? Can J Bot 82:1089–1109

Jones MD, Durall DM, Harniman SMK, Classen DC, Simard SW (1997) Ectomycorrhizal diversity of *Betula papyrifera* and *Pseudotsuga menziesii* seedlings grown in the greenhouse or in single-species and mixed plots in southern British Columbia. Can J Forest Res 27:1872–1889

Jones MD, Durall DM, Cairney JWG (2003) Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytol 157:399–422

Jumpponen A (2001) Dark septate endophytes—are they mycorrhizal? Mycorrhiza 11:207–211

Jurgensen MF, Harvey AE, Graham RT, Page-Dumroese DS, Tonn JR, Larsen MG, Jain TB (1997) Impacts of timber harvesting on soil

organic matter, nitrogen, productivity and health of inland Northwest forests. For Sci 43:234–251
Klironomos JN (2003) Variation in plant response to native and exotic mycorrhizal fungi. Ecology 84:2292–2301
Koide RT (1991) Nutrient supply, nutrient demand and plant response to mycorrhizal infection. New Phytol 117:365–386
Kolotelo D, van Steenis E, Peterson M, Bennett R, Trotter D, Dennis J (2001) Seed handling guidebook. British Columbia Ministry of Forests, Tree Improvement Branch, Victoria, Canada
Levang-Brilz N, Biondini ME (2002) Growth rate, root development and nutrient uptake of 55 plant species from the Great Plains Grasslands, USA. Plant Ecol 165:117–144
Lloyd D, Angrove K, Hope G, Thompson C (1990) A Guide to Site Identification and interpretation for the Kamloops Forest Region. Victoria, B.C.: Research Branch, B.C. Ministry of Forests
Mandyam K, Jumpponen A (2005) Seeking the elusive function of the root-colonizing dark septate endophytic fungi. Stud Mycol 53:173–189
Mandyam K, Jumpponen A (2008) Seasonal and temporal dynamics of arbuscular mycorrhizal and dark septate endophytic fungi in a tallgrass prairie ecosystem are minimally affected by nitrogen enrichment. Mycorrhiza 18:145–155
Marx DH, Hatch AB, Mendicino JF (1977) High soil fertility decreases sucrose content and susceptibility of loblolly pine to ectomycorrhizal infection by *Pisolithis tinctorius*. Can J Bot 55:1569–1574
Mason PA, Wilson J, Last FT (1983) The concept of succession in relation to the spread of sheathing mycorrhizal fungi on inoculated tree seedlings growing in unsterile soils. Plant Soil 71:247–256
McCune B, Grace JB (2002) Analysis of ecological communities. MjM Software Design, Gleneden Beach
Nara K (2006) Ectomycorrhizal networks and seedling establishment during early primary succession. New Phytol 169:169–178
Newsome TA, Sutherland DC, Vyse A (1991) Establishing Douglas-fir plantations in the dry belt of interior British Columbia. In: Baumgartner DM, Lotan JE (eds) Interior Douglas-fir: the species and its management. Washington State University, Cooperative Extension Service, Seattle, pp 227–233
Newton AC (1992) Towards a functional classification of ectomycorrhizal fungi. Mycorrhiza 2:75–79
Parke JL, Linderman R, Black C (1983) The role of ectomycorrhizas in drought tolerance of Douglas-fir seedlings. New Phytol 95:83–95
Perry DA, Amaranthus MP (1997) Disturbance, recovery and stability. In: Kohm KA, Franklin JF (eds) Creating a forestry for the 21st century: the science of ecosystem management. Island Press, Washington, pp 31–56
Perry DA, Amaranthus MP, Borchers JG, Borchers SL, Brainerd RE (1989) Bootstrapping in ecosystems. Bioscience 39:230–237
Reid CPP, Kidd FA, Ekwebelam SA (1983) Nitrogen nutrition, photosynthesis and carbon allocation in ectomycorrhizal pine. Plant Soil 71:415–432
Rillig MC (2004) Arbuscular mycorrhizas and terrestrial ecosystem processes. Ecol Lett 7:740–754
Rincón A, Parladé J, Pera J (2005) Effects of ectomycorrhizal inoculation and the type of substrate on mycorrhization, growth and nutrition of containerized *Pinus pinea* L. seedlings produced in a commercial nursery. Ann For Sci 62:817–822
Runion B, Mitchell RJ, Rogers HH, Prior SA, Counts TK (1994) Effects of nitrogen and water limitation and elevated atmospheric CO_2 on ectomycorrhiza of longleaf pine. New Phytol 137:681–689
Schwartz MW, Hoeksema JD (1998) Specialization and resource trade: biological markers as a model of mutualisms. Ecology 79:1029–1038
Simard SW, Jones MD, Durall DM, Hope GD, Stathers RJ, Sorensen NS, Zimonick BJ (2003) Chemical and mechanical site preparation: effects on *Pinus contorta* growth, physiology and microsite quality on grassy, steep forest sites in British Columbia. Can J For Res 33:1495–1515
Simard SW, Hagerman SM, Sachs DL, Heineman JL, Mather WJ (2005) Conifer growth, *Armillaria ostoyae* root disease and plant diversity responses to broadleaf competition reduction in temperate mixed forests of southern interior British Columbia. Can J For Res 35:843–859
Stack RW, Sinclair WA (1975) Protection of Douglas-fir seedlings against *Fusarium* root rot by a mycorrhizal fungus in the absence of mycorrhiza formation. Phytopathology 65:468–472
Swaty RL, Deckert RJ, Whitham TG, Gehring CA (2004) Ectomycorrhizal abundance and community composition shifts with drought: predictions from tree rings. Ecology 85:1072–1084
Teste FP, Simard SW (2008) Mycorrhizal networks and distance from mature trees alter patterns of competition and facilitation in dry Douglas-fir forests. Oecologia 158:193–203
Teste FP, Schmidt MG, Berch SM, Bulmer C, Egger KN (2004) Effects of ectomycorrhizal inoculants on survival and growth of interior Douglas-fir seedlings on reforestation sites and partially rehabilitated landings. Can J For Res 34:2074–2088
Teste FP, Simard SW, Durall DM (2009) Role of mycorrhizal networks and tree proximity in ectomycorrhizal colonization of planted seedlings. Fungal Ecol 2:21–30
Treseder KK, Allen MF (2002) Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. New Phytol 155:507–515
Twieg BD, Durall DM, Simard SW (2007) Ectomycorrhizal fungal succession in mixed temperate forests. New Phytol 176:437–447
Twieg BD, Durall DM, Simard SW, Jones MD (2009) Influence of soil nutrients on ectomycorrhizal communities in a chronosequence of mixed temperate forests. Mycorrhiza 19(5):305–316
Yang CS, Korf RP (1985) A monograph of the genus *Tricharina* and of a new, segregate genus, *Wilcoxina* (Pezizales). Mycotaxon 24:467–531