Mycorrhizal Fungus Communities of Douglas-fir (*Pseudotsuga menziesii*) Seedlings and Trees: Effects of Proximity to Residual Trees

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Abstract

Mycorrhizal Fungus Communities of Douglas-fir (*Pseudotsuga menziesii*) Seedlings and Trees: Effects of Proximity to Residual Trees

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Chair of the Supervisory Committee

Professor Robert L. Edmonds
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The influence of mature Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees in partially harvested stands on seedling growth and colonization by ectomycorrhizal fungi (EMF) is not well understood. To investigate this, Douglas-fir seedlings were planted near (<6 m) and far (>16 m) from 44- to 72-year-old residual Douglas-fir trees at three sites in western Washington, USA. Seedling stem growth was measured from 1998 through 2003 and root growth from 1998 to 2000. Growth of seedlings potted in soils gathered near each residual tree was also examined in a greenhouse at the University of Washington. Monthly soil cores were collected near residual trees, and residual tree roots were sampled by partially excavating the root system. Colonized roots were examined by morphotyping, PCR-RFLP, and sequence analysis of the internal transcribed spacer (ITS) and other ribosomal RNA genes.

Seedlings <6 m from residual trees had significantly higher root to shoot biomass ratios. Stem growth was greater for seedlings <6 m from trees at a site with minimal understory, but slower at sites with more developed understory. EMF abundance was related positively to both root and stem growth in 1998 but was negatively related to stem growth in 1999.

Seedlings <6 m from residual trees averaged 4.1 EMF taxa per seedling and had a total of 47 taxa compared to 3.3 taxa per seedling and 38 total taxa for >16 m seedlings. *Rhizopogon* spp. were dominant on both <6 m and >16 m seedlings. EMF taxa of residual trees, including *Russula* spp. (particularly *R. nigricans*), *Tylospora* spp., *Tomentella* spp., and *Boletus zelleri*, were more frequent on <6 m seedlings than on >16 m seedlings. The EMF community of >16 m seedlings was more similar to that of greenhouse seedlings.

Russula nigricans, Boletus zelleri, Clavulina, and *Rhizopogon parksii* were common in soil cores. EMF abundance and diversity were highest at the less recently harvested site, and increased from 1998 to 1999 at all sites.

Proximity to residual trees increases root growth and EMF abundance and diversity of seedlings. Residual trees may accelerate the establishment of mycorrhizal communities associated with mature forests.

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DEDICATION

To my mother,
for nourishing my wonder at the beauty of life.

You are missed.

Chapter 1. Introduction

Maintenance of biodiversity has increasingly become a priority in management of both public (FEMAT 1993; WFPB 1995) and private (WFPA 1995) forest lands. Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) forest management in the Pacific Northwest has been transformed in the last decade due to concerns about declining biodiversity. A management strategy that has been widely adopted in place of clearcutting is partial harvesting which retains some living trees (Kohm and Franklin 1997). Residual trees may harbor forest-dependent species while the young forest regenerates (North et al. 1996). This might be of particular importance for mutualistic ectomycorrhizal fungi (EMF) associated with conifer trees. It is necessary to evaluate the effectiveness of partial retention for promoting diversity of EMF communities in regenerating stands.

It is not known how EMF taxa associated with isolated residual trees respond to the environmental changes resulting from harvesting. The effectiveness of residual trees in harboring forest-associated EMF taxa and the effect of proximity to residual trees on Douglas-fir seedling growth and mycorrhizal status has not been investigated in the Pacific Northwest.

Globally, ectomycorrhizal fungal communities are not well characterized. Some detailed studies have recently been published describing ectomycorrhizas from a variety of forest types in North America and Europe, summarized in reviews by Taylor et al. (2000), Dahlberg (2001), and Horton and Bruns (2001). Neverthess, information on the ectomycorrhizal taxa associated with Douglas-fir is limited. This study provides a detailed description of the ectomycorrhizal communities associated with Douglas-fir seedlings and trees. The use of molecular methods in combination with morphotyping has allowed the majority of ectomycorrhizal fungus taxa collected from roots to be tentatively identified to the species level, and identified with confidence to the genus level.

The dissertation is structured in six chapters plus three appendices. The first chapter introduces the study. The second chapter, a review of pertinent literature, places the study within the context of other research. A detailed treatment of other community-level studies of mycorrhizal fungi includes a global comparison of EMF taxa observed in various forested regions. Chapters three through five are written as stand-alone papers. Finally, chapter six summarizes the results presented in chapters three through five, and presents overall conclusions.

The first objective of this study, addressed in chapter three, was to assess the spatial influence of residual trees on growth and mycorrhizal colonization of Douglas-fir seedlings. The following hypotheses were tested: (i) seedlings near residual trees have higher levels of mycorrhizal colonization, (ii) seedlings near residual trees have higher levels of mycorrhizal diversity per seedling than a) seedlings growing beyond the root zone of residual trees and b) seedlings exposed to soil propagules but deprived of root connections, (iii) seedlings growing near residual trees experience growth inhibition due to shading or other competitive interactions, and (iv) seedling growth rates are influenced by mycorrhizal status.

The second objective of this study, addressed in chapter four, was to assess the spatial influence of residual trees on EMF communities of Douglas-fir seedlings. The following hypotheses were tested: (i) the EMF community of seedlings growing near isolated mature trees will be more diverse than that of seedlings far from trees, (ii) EMF taxa associated with mature trees will be more common on seedlings near mature trees, and (iii) EMF taxa able to colonize seedlings from soil propagules (i.e., those present on greenhouse seedlings) will be more common on seedlings far from mature trees.

The third objective of this study, addressed in chapter five, was to assess changes of EMF communities of residual Douglas-fir trees in harvested stands. Specifically, the goal was to determine (i) annual changes in the EMF community after harvesting, (ii) changes from spring to fall in EMF communities, and (iii) how soil characteristics are related to the distribution of EMF taxa.

Chapter 2. Literature Review

2.1 Douglas-fir forest management in the Pacific Northwest

Lower elevation forests in the Pacific Northwest are commonly dominated by Douglas-fir, a tree species that flourishes under a natural fire regime characterized by infrequent, extensive stand-replacing fires. In the mesic forests of the western Cascade mountain range, Douglas-fir fills an early-successional niche, germinating and growing rapidly in the bright light conditions of areas recently cleared by fire, but unable to regenerate in the shade of a closed canopy mature forest. As a result, older stands of Douglas-fir are gradually replaced by a climax species, commonly western hemlock (*Tsuga heterophylla (Raf.) Sarg.*), in the absence of fire or other large-scale disturbance. Because natural fire return intervals in this region are generally shorter than the average life-span of Douglas-fir, forests in this region commonly remain dominated by Douglas-fir indefinitely. The natural disturbance regime, including fire, windthrow, and mortality due to insects and pathogenic fungi, maintains a mosaic across the landscape of patches of younger forest within a matrix of mature to late-successional forest.

2.1.1 Impacts of clearcutting

As an important commercial tree species valued by the timber industry for its rapid growth and straight grain, Douglas-fir has been extensively harvested in the Pacific Northwest over the past century. Early harvesting methods often selected the largest or most valuable trees, but this was replaced by clearcutting or clearfelling. Accidental fires or broadcast burning reduced woody debris and competition from the understory, which had the advantage of encouraging Douglas-fir natural regeneration or enhancing survival of planted seedlings after harvesting.

Widespread clearcutting on public and private lands over the last century has not only shifted the landscape mosaic from predominantly late-successional forest to younger second- or third-growth forests, but has also changed the pattern of the landscape

(Franklin et al. 1986). Fires often result in large contiguous stands. In contrast, harvesting in the Pacific Northwest has occurred predominantly in smaller patches, creating a highly fragmented landscape which is characterized by highly contrasting edges between older and younger stands. The edge between forest and clearcut represents a zone of interaction in which edge effects may extend both into the forest and into the clearcut (Sparks 2004). Forests bordering on clearcuts experience differences in light exposure and microclimate which can result in tree mortality due to windthrow (Esseen 1994), increased growth rates of dominant trees, and shifts in understory tree species composition (Chen et al. 1992). Soil processes including decomposition rates and nitrogen release can be impacted by changes in soil moisture and temperature which commonly occur at forest-clearcut boundaries (Edmonds et al. 2000). Decomposer and mycorrhizal fungi demonstrate characteristic distribution patterns, revealed by sporocarp surveys along forest to clearcut transects (Sparks 2004). In old-growth forests bordering on 10- to 15-year-old clearcuts, edge effects extended up to 137 m into the forest (Chen et al. 1992). For organisms that require interior forest habitat, the effective size of remaining late-successional forest patches can be drastically reduced by edge effects.

In addition to decreasing the total area of late-successional forest and minimizing the effectiveness of remaining patches through edge effects and fragmentation, clearcutting has also altered the composition of regenerating forests. Fires and other natural disturbances often leave patches of standing trees, living or dead, which can serve as biological legacies (Franklin 1990). Residual trees may play a role both in the short term by ameliorating conditions which allow other organisms to persist through the early stages of forest regeneration, as well as in the long term, by increasing the structural complexity of the regenerating stand (Perry et al. 1989a; Franklin 1992a; Franklin 1992b). Unburned patches often contain advance regeneration of other conifer species such as western hemlock, resulting in higher tree diversity. In stands with trees 65-125 years old regenerating after natural fires, Traut and Muir (2000) found that those stands which had patches of older remnant trees had a higher percentage of regenerating western hemlock. While understory species richness did not differ between stands with and without remnant trees, the herb and shrub community composition was correlated with the percentage of western hemlock in the regenerating overstory (Traut and Muir 2000). Therefore, patches of remnant trees might maintain a higher understory diversity at the landscape level.

2.1.2 Alternative harvesting methods

Mounting concern over the impacts of clearcutting have prompted both private (WFPA 1995) and public forest managers (FEMAT 1993; WFPB 1995) to adopt new harvesting practices which include, to varying degrees, provisions for retention of green trees, snags, and logs. Combined with increased attention to sustainability and landscape-level issues of connectivity and fragmentation, these measures have been referred to as new perspectives, new forestry, or ecosystem management (Franklin 1992a; Franklin 1993).

Partial cutting, or green tree retention, has been proposed to more closely mimic the structure of a stand regenerating from a natural fire or wind disturbance, however the efficacy of these recently-adopted methods have not yet been thoroughly evaluated. Partial cutting can be subdivided into two categories: dispersed green tree retention with more-or-less evenly spaced trees or aggregated retention of clusters of trees and snags, for example, as stream buffers (Franklin 1992a). A comparative study of clearcuts, dispersed green tree retention, and uncut 65-year-old stands in the Cedar River watershed found higher understory plant diversity in the retention stands than in either the forest or clearcut, six years after harvesting (North et al. 1996). The plant community in the retention stands was intermediate in that it included species characteristic of the clearcut as well as forest species (North et al. 1996). Another study in the same watershed found few significant differences in air and soil temperature, soil moisture, and nitrogen transformations between clearcuts, dispersed retention stands, and forest, however, soil microbial biomass was found to be higher within one meter of residual trees than six meters from residual trees (Barg and Edmonds 1999). These studies of initial responses to partial retention provide some indication that dependent plant and microbial communities may be influenced by residual trees over the short term, but clearly more studies are required.

Another issue of concern which is particularly pressing in dispersed retention is how Douglas-fir seedling growth and survival will be affected by partial shading. Edmonds et al. (2000) found no significant difference in seedling survival over the first growing season, in seedlings planted within one meter of, or over six meters from retention trees. In more harsh environments (hot in the summer, cold in the winter) seedling survival can be enhanced by creating shelterwoods. This silvicultural treatment has been applied successfully with a longer historical record, but it differs from dispersed retention in that retained trees are not allowed to persist throughout the next rotation, but are instead cut down after seedlings are well established. Close proximity to retention trees might be expected to have a negative effect on longer-term growth and survival of seedlings or saplings as the stand regenerates, but the effect may depend on the density of the retention trees. In mature Douglas-fir stands thinned to a basal area of 16-31 m^2/ha, planted Douglas-fir seedlings had only 10 to 35% survival rates, with lowest survival rates in stands with the highest density (Brandeis et al. 2001; Brandeis et al. 2002).

2.2 Douglas-fir seedling growth and mycorrhizal fungi

2.2.1 Factors influencing seedling growth

The growth rate of Douglas-fir seedlings at a given site depends on a variety of factors, including initial size of the seedling and its root system (Lopushinsky and Beebe 1976; van den Driessche 1992), degree of intra- and interspecific competition (Newton and Cole 1991; Shainsky et al. 1992; Tesch et al. 1993), and herbivory (Brandeis et al. 2002). The size of the seedling at planting affects the surface area of foliage and thus the supply of photosynthate, the size of the root system controls access to water and nutrients, and competition reduces availability of light, water, or nutrients. While Douglas-fir is commonly considered to be shade-intolerant, and therefore might be assumed to be most limited by light, it can be difficult to separate the effects of competition for light from competition for belowground resources. Studies of Douglas-fir seedlings grown in pots (Khan et al. 2000) and in raised beds (St. Clair and Sniezko 1999) have demonstrated that shading up to 75% results in decreases in total seedling biomass production but increases in height growth over the first 1-2 years. The increase in height appears to occur both through partitioning of resources to the shoot more than the root system, reflected by an increase in the ratio of shoot biomass to root biomass, as well as by shifting more resources to vertical growth instead of diameter increase or branch production (St. Clair and Sniezko 1999).

These results illustrate a critical caveat in interpreting growth effects; namely, the importance of considering the root system as well as the shoot system. Increases in aboveground biomass may merely reflect shifts in allocation rather than changes in true productivity. In one of the few studies that addresses both above-ground and below-ground productivity in Douglas-fir forests, Keyes and Grier (1981) compared 40-year-old forests whose productivity differed due to soil type. While aboveground productivity was higher on the high productivity soils, the total productivity was similar among sites. Differences were primarily due to increased belowground productivity, with forests at the poorer site producing nearly double the root biomass. Douglas-fir had a much greater investment in fine roots on the poorer site, allocating over 36% of total production to fine roots in comparison to only 8% at the higher productivity site (Keyes and Grier 1981). Vogt et al. (1987) also found significantly higher fine root biomass in low-productivity naturally regenerated Douglas-fir stands when compared to higher productivity stands.

2.2.2 Attributes of mycorrhizal fungi

Another important factor to consider is the role of mycorrhizal fungi in providing access to belowground resources. Although Douglas-fir seedlings can be grown axenically in the lab, and there are reports of naturally-seeded western hemlock (Christy et al. 1982), lodgepole pine (*Pinus contorta)*, and subalpine fir (*Abies lasiocarpa*) seedlings (Miller et al. 1992) surviving up to one growing season without mycorrhizal fungi, in field settings Douglas-fir seedlings require mycorrhizal fungi for survival and growth (Molina et al. 1992).

A large number of fungal taxa distributed across several fungal phyla have developed the ability to form symbiotic associations with the roots of plants. These mycorrhizal ("fungus-root") associations were probably a prerequisite to colonization of land by primitive plants and preceded the development of roots (Brundrett 2002). Mycorrhizas occur in a variety of forms, differing by the degree of host plant tissue invasiveness exhibited by the fungus. The first to evolve were arbuscular mycorrhizas, formed by fungi in the newly acknowledged phylum Glomeromycota (Schussler et al. 2001). Arbuscular mycorrhizas have highly branched structures termed arbuscules which form inside the host plant's root cortical cells as invaginations of the plasma membrane (Smith et al. 1994). Arbuscular mycorrhizal fungi do not noticeably modify the morphology of the host plant's roots, and uptake of water and nutrients occurs via a diffuse network of hyphae extending out from the root (Marschner and Dell 1994).

While arbuscular mycorrhizas predominate on some conifers such as cedars, junipers, and yew trees (members of the Cupressaceae and Taxodiaceae), members of the Pinaceae are dependant on ectomycorrhizal fungi, which form a sheath or mantle around the exterior of fine roots, and induce characteristic branching patterns in root tips. Ectomycorrhizal fungal hyphae grow within the cortex of the host root, forming a structure called the Hartig net around the root cells, but hyphae do not penetrate the cortical cell wall as occurs with arbuscular mycorrhizas. Nutrient uptake can occur through the fungal mantle sheathing the fine root tips, but probably occurs mostly through emanating hyphae (Marschner and Dell 1994) which vary widely and characteristically between fungal taxa. Agerer (2001) distinguishes five exploration strategies ranging from contact exploration (few or no emanating hyphae) to long-distance exploration types, which produce bundles of hyphae called rhizomorphs which are differentiated for long distance transport and can extend up to several decimeters in length. The fungi which form ectomycorrhizas are polyphyletic, including multiple clades within the Basidiomycota as well as some in the Ascomycota, as the mycorrhizal ability appears to have evolved multiple times throughout evolutionary history (Hibbitt et al. 2000).

As a member of the Pinaceae, Douglas-fir predominantly forms ectomycorrhizas. Arbuscular mycorrhizas may co-occur with ectomycorrhizas, especially in seedlings, but the ecological significance of this has not been measured (Cazares and Trappe 1993; Cazares and Smith 1996; Smith et al. 1998). Douglas-fir is characterized by an unusually broad host receptivity, as it is capable of hosting over 2000 fungal taxa (Trappe 1977). It is important to distinguish between host receptivity and the host specificity of each of the fungal associates. There are an unusually high number of highly specific fungal associates which appear to be restricted to trees in the genus *Pseudotsuga*, an estimated 250 of the 2000 cited above (Molina et al. 1992), many of which are fungi in the genus *Rhizopogon* (Molina and Trappe 1994). Of the remainder, many fungal associates have relatively broad host ranges. Molina et al. (1992) estimate that over 70% of Douglas-fir associates are also compatible with species in the pine genus, and numerous field studies have observed fungal taxa identified on root tips of more than one host tree species in a given stand (Simard et al. 1997a; Horton and Bruns 1998a; Kranabetter et al. 1999; Massicote et al. 1999).

This raises the possibility that trees of different species could commonly be interconnected through their mycorrhizal fungi. Laboratory studies have demonstrated that common mycelial networks among seedlings have the capacity to transfer carbon and nitrogen from one to the other (Finlay and Read 1986; Arnebrant et al. 1993; Simard et al. 1997b). Simard et al. (1997c) demonstrated using carbon isotopes that ectomycorrhizal paper birch (*Betula papyrifera*) seedlings transferred net carbon to Douglas-fir seedlings, while arbuscular mycorrhizal western red cedar (*Thuja plicata*) seedlings had a much lower carbon gain, presumably through soil solution pathways. Transfer could have been driven by either a carbon or a nitrogen gradient, but the authors speculated that carbon could have been transferred in amino acids, driven by the nitrogen gradient (Simard et al. 1997c). A large-scale field study performed by Booth (2004) found that, in the absence of competition from mature tree roots, eastern white pine

(*Pinus strobus*) seedlings had increased needle production when common mycorrhizal networks were allowed to form. Further studies are needed to determine under what environmental conditions common mycorrhizal networks play a significant physiological role.

2.2.3 Benefits and costs of mycorrhizal fungi

Regardless of whether seedlings are acquiring physiologically significant quantities of carbon or nitrogen from other trees via common mycelial networks, it is clear that mycorrhizal fungi are crucial in accessing belowground resources. It is well documented that ectomycorrhizal fungi can take up and transfer phosphorous, ammonium, nitrate, potassium, calcium, and iron to their host plant (Marschner and Dell 1994; Smith et al. 1994). In addition, some ectomycorrhizal taxa produce siderophores or chelating agents while others produce extracellular enzymes which can break down organic substrates, mobilizing organic forms of nitrogen or phosphorus (Read and Perez-Moreno 2003).

Mycorrhizal fungi can reduce water stress in their host plants through increased uptake, storage, and transport of water (Parke et al. 1983), as well as by removing nutrient limitations and potentially allowing for greater development of root systems (Bledsoe 1986). With their narrow hyphae, mycorrhizal fungi can access tightly-held water in small pores and crevices of soil particles and aggregates, where the larger-diameter plant roots cannot penetrate. It is difficult to quantify the extent of the fungal mycelium in the soil, but well-developed mycelia could store substantial quantities of water, especially those fungi that form dense proliferations of hyphae known as mycelial mats or produce abundant rhizomorphs. Mycorrhizal colonization by certain fungi such as *Cenococcum geophilum* also appears to increase the survival of roots experiencing moisture stress (Pigott 1982).

While mycorrhizal associations provide clear benefits, there are also costs to the plant in the form of sometimes substantial investments of photosynthate provided to the fungus. It is not uncommon to observe neutral or negative correlations between degree of mycorrhizal colonization and growth attributes of the host plant, especially in greenhouse or nursery studies where nutrients and/or water are not limiting (Harley and Smith 1983). Even in field settings, seedlings may not always benefit from high mycorrhizal colonization rates, at least in the short term. While rapid mycorrhizal colonization appears to be essential for seedling survival, especially in harsh or disturbed environments (Trappe 1977; Perry et al. 1987), the relationship between amount of mycorrhizal colonization and seedling growth can be quite inconsistent from study to study (Jones et al. 2003). Field observations cannot establish causation, but it is interesting that seedlings with the largest number of ectomycorrhizas per gram of root sometimes have reduced height, diameter, and/or total biomass. This was observed for naturally regenerated seedlings in a mixed conifer forest in Montana (Harvey et al. 1987) and for red pine (*Pinus resinosa*) seedlings planted in Michigan clearcuts (Wu et al. 1993). In contrast, Harvey *et al.* (1991) found positive correlations between Douglas-fir seedling growth and numbers of ectomycorrhizal tips, through the second season after outplanting.

2.2.4 Effects of mycorrhizal inoculation on seedling growth

Most research on the effects of mycorrhizas on seedling growth has come from inoculation studies. Inoculation allows for comparison of the effects of one or a few fungal taxa, but it is important to keep in mind that these studies usually occur in simplified systems and are by necessity confined to fungi that establish readily from spores or other propagules. In addition, interpretation of the results can be complicated by inconsistent measures of growth. Measurement of above and belowground biomass provides the most information, but requires destructive sampling of seedlings, is labor intensive, and rapidly becomes unrealistic as seedlings mature. Height and diameter measurements are easy to obtain, but only reflect aboveground production, and therefore will not detect changes in allocation of resources from shoot to root which commonly occur with mycorrhizas (Marx et al. 1991). Seedlings must balance allocation of photosynthate to nutrient acquisition structures (roots and their associated mycorrhizal fungal mycelia) with allocation to aboveground shoot systems for competition for light.

Responses of seedlings to inoculation often evaluate the effectiveness of specific taxa as opposed to the effectiveness of mycorrhizas in general, since non-inoculated seedlings commonly acquire "volunteer" mycorrhizal fungi such as *Thelephora terrestris*. In one study, for Douglas-fir seedlings in pots, inoculation with *Laccaria laccata* and *Rhizopogon vinicolor* produced growth stimulation, while *Hebeloma crustuliniforme* reduced seedling growth relative to uninoculated controls (Perry et al. 1987). McAfee and Fortin (1988) also found that *Laccaria bicolor* inoculation stimulated seedling growth for *Pinus banksiana* grown in sterile peat/vermiculite soils over inoculation by *Pisolithus tinctorius* or *Hebeloma cylindrosporum* alone or in combination with *L. bicolor*. Another study found no significant inoculation effect by *Laccaria bicolor* on biomass of container-grown Douglas-fir seedlings fertilized with various levels of nitrogen (Gagnon et al. 1995). Pre-inoculation followed by outplanting also reveals a complex pattern of results. Douglas-fir seedlings pre-inoculated with *Laccaria bicolor* and outplanted in France, where Douglas-fir is exotic and therefore native inoculum would be expected to be limited, had a 160% increase in root biomass and a 239% increase in shoot biomass compared to uninoculated controls (Villeneuve et al. 1991). Likewise, Douglas-fir pre-inoculated with *Rhizopogon vinicolor* and *R. colossus* and outplanted in southwest Oregon had significantly greater height, diameter, and total biomass than uninoculated controls (Castellano and Trappe 1985). In contrast, Douglas-fir seedlings pre-inoculated with *Rhizopogon vinicolor* had no significant difference in growth one growing season after outplanting on Vancouver Island, even though *R. vinicolor* persisted on the roots (Berch and Roth 1993), and inoculation of Douglas-fir with *Hebeloma crustuliniforme* and *Laccaria laccata* resulted in a substantial reduction in biomass compared to uninoculated controls in dry, recently burned sites in the eastern Cascade range of Washington state (Bledsoe and Tennyson 1982).

The inconsistent pattern of results reflects the dynamic nature of the mycorrhizal relationship. Seedling needs may change both seasonally and annually, combining with differences in physical environment or climate to define a unique balance of cost and benefit for each different fungal taxon over time. For example, inoculation with

Rhizopogon may not confer much advantage under normal conditions, but could pay off during periods of drought when its abundant rhizomorphs can rapidly transport available water (Parke et al. 1983). Harvey et al. (1991) found a positive correlation, in the dry summer months only, between Douglas-fir seedling growth and number of ectomycorrhizal tips. Mycorrhizal fungi may also provide protection from soil pathogens (Fitter and Garbaye 1994) and stimulate associative nitrogen-fixing bacteria (Chanway and Holl 1991). Potential benefit of a fungal taxon to a seedling can depend on competitive interactions. Shading by other trees can reduce the potential for production of photosynthate enough that costs of mycorrhizas exceed benefits (Johnson et al. 1997). Mycorrhizal fungi may also modify competition for nutrients. For Douglas-fir seedlings grown in common pots with ponderosa pine, inoculation with *Laccaria laccata* resulted in a release of mutual growth inhibition, presumably due to increased uptake of N and P by Douglas-fir at the expense of luxury consumption by Ponderosa pine seedlings (Perry et al. 1989b).

Ultimately, the type of fungal associate appears to be more important than the number or density of mycorrhizal tips for seedlings. Fungal taxa vary in their abilities to take up organic and inorganic forms of nitrogen, phosphorous, and other nutrients (van der Heijden and Kuyper 2003), and tolerance to moisture stress (Bledsoe 1986). A diverse array of mycorrhizal fungi may confer a potential advantage since different fungi may offer unique physiological abilities to access organic or inorganic pools of nutrients (Read and Perez-Moreno 2003) or may be specialized for different depths in the soil profile (Sylvia and Jarstfer 1997; Dickie et al. 2002b), or for different types of soil substrates (Kropp 1982; Harvey et al. 1987; Landeweert et al. 2003; Rosling et al. 2004). Effects on seedlings are not limited to changes in nutrition or productivity. A greenhouse study found that Douglas-fir seedlings adopted a different root morphology when inoculated with *Rhizopogon vinicolor*, resulting in shorter roots without changing root or shoot biomass relative to uninoculated controls or to seedlings inoculated with *Laccaria laccata* (Dosskey et al. 1992). Similar results were obtained with seedlings inoculated with *Laccaria bicolor* and outplanted in France. *Laccaria* persisted on older roots and spread to new roots over the first two seasons after outplanting, while stimulating increased root length and root development at depths greater than 20 cm compared to uninoculated seedlings (Villeneuve et al. 1991). Caution must be used in extending these findings to other tree species, since the opposite effect was observed in creeping willow (*Salix repens*) seedlings, with *Laccaria* inoculation associated with less root development than any of the other fungal taxa tested (van der Heijden and Kuyper 2003). It remains to be explored how such anatomical changes may affect survival and growth over the long term.

Studies which compare combinations of inoculated fungi address the importance of fungal diversity in seedling growth. An increase in the number of species inoculated has been shown to result in growth stimulation in several studies (McAfee and Fortin 1988; Jonsson et al. 2001; Jones et al. 2003). Interpretation of these results is often confounded by changes in numbers of root tips colonized, as well as by potential sampling effects, in which a larger sample of fungi is more likely to include a "star player" in terms of growth stimulation (Wardle 1999). For example, McAfee and Fortin (1988) saw increases in seedling growth with inoculation by two and three fungal species, but the difference appeared to be caused by the growth-stimulating effects of *Laccaria bicolor* in the mixed samples. Perry et al. (1989b) also found *Laccaria laccata* to be responsible for increased growth of seedlings inoculated with multiple fungi. Jonsson et al. (2001) controlled for potential sampling effects in their greenhouse study of growth of European white birch (*Betula pendula*) and scotch pine (*Pinus sylvestris*) inoculated by single species or combinations of fungal taxa. Fungal inoculations of two, four, or eight species could more than double seedling growth when compared to single species inoculations, but the effect depended both on the tree species and on the fertility of the soil substrate. For the pine seedlings, shoot and root biomass was negatively correlated with species richness in high fertility soils only, while for the birch seedlings, shoot and root biomass were positively correlated with species richness in the low fertility soils only (Jonsson et al. 2001). In a similar greenhouse study, inoculation of gray birch (*Betula populifolia*) with combinations of four species of ectomycorrhizal fungi resulted in an increase in root colonization and a shift in allocation from shoot to root when compared to single-species inoculations (Baxter and Dighton 2001). The inconsistency of the relationship between fungal diversity and growth may be partially due to the simplified growing conditions of greenhouse studies. Effects of diversity might be expected to be enhanced by the diverse microhabitats and temporal changes which are associated with natural soils and ecosystems, but more research in field settings is needed (Jones et al. 2003).

Additional support for the importance of a diverse mycorrhizal community comes from studies of the effect of soil transfers on seedling growth, which often result in increased mycorrhizal species richness. General applicability of these studies is limited, however, because they occur in situations where normal mycorrhizal colonization has been reduced by human impacts. For example, Berman and Bledsoe (1998) found that soil transfer from soils near oak trees significantly increased ectomycorrhizal diversity of oak seedlings grown in agricultural soils where natural ectomycorrhizal inoculum was assumed to be lacking. Increases in ectomycorrhizal diversity were correlated with increased shoot growth and decreased root growth, the opposite of the pattern observed by Baxter and Dighton (2001). Due to the field setting, Berman and Bledsoe (1998) were not able to control for possible sampling effects. In a series of studies of clearcuts in Oregon where seedling regeneration had been unsuccessful, failure of seedlings to survive may have been related not only to reduced mycorrhizal inoculation potential (Pilz and Perry 1984), but also to changes in soil structure (Borchers and Perry 1990). Soil transfers from plantation soils, but not from natural forest soils, resulted in increased Douglas-fir seedling survival and growth. However, the effect appeared to be mediated in part by soil grazers which by consuming soil fungi may stimulate nutrient release, not solely by increased mycorrhizal inoculation (Colinas et al. 1994).

2.3 Community ecology of ectomycorrhizal fungi

2.3.1 Methods for studying ectomycorrhizal communities

The field of mycorrhizal community ecology has advanced tremendously in the past decade due to the advent of DNA based methods for identifying fungi on root tips, building upon the traditional morphological approach. These recent studies have augmented a long history of community analysis based on sporocarp production which has yielded valuable insights into fungus host specificity, succession, and response to disturbances such as forest harvesting, fire, and nitrogen deposition (Hering 1966; Last et al. 1984; Bills et al. 1986; Dighton et al. 1986; Villeneuve et al. 1989; Arnolds 1991; Claridge et al. 1993; Termorshuizen 1993; Waters et al. 1994; Visser 1995; States and Gaud 1997; Countess et al. 1998; Jumpponen et al. 1999; Smith et al. 1999; Claridge et al. 2000; Kranabetter and Kroeger 2001; Smith et al. 2002; Nara et al. 2003; Shaw et al. 2003). Unfortunately, many studies have revealed a fundamental disconnect between sporocarp production and the underlying community composition of fungi on the roots (Gardes and Bruns 1996; van der Heijden et al. 1999; Jonsson et al. 2000; Yamada and Katsuya 2001), although sometimes changes in sporocarp production are related to changes in the mycorrhizal community on the roots (Avis et al. 2003). The discrepancy between sporocarp production and root colonization is partly explained by the wide distribution and abundance of resupinate thelephoroid fungi (e.g., *Tomentella, Pseudotomentella*) whose sporocarps are small, inconspicuous, and often overlooked in surveys of fungal sporocarps (Koljalg et al. 2000).

Molecular methods targeting the fungal ribosomal RNA genes have become standard practice. Fungal specific primers (White et al. 1990) are used with the polymerase chain reaction (PCR) to amplify sufficient quantities of DNA for restriction fragment length polymorphism (RFLP) analysis or for direct sequence determination. RFLP analysis uses restriction enzymes, isolated from bacteria, that cut DNA at locations defined by short motifs of 4-6 bases. For example, if the DNA fragment of interest has the restriction enzyme recognition site, it will be cut into two fragments which can be detected using gel electrophoresis. To determine the identity of an unknown fungus on a mycorrhizal root tip, the RFLP pattern of the sample must be matched to RFLP patterns from known taxa, which are generated from sporocarps. RFLP analysis is fairly inexpensive and can be useful for sorting large numbers of samples, but there are some limitations to the method. Because the RFLP pattern depends on the restriction enzymes chosen by a particular researcher, RFLP libraries are often generated anew for each researcher or project. Frequently, a substantial proportion of sample RFLP patterns cannot be matched to collected sporocarps, especially in ecosystems with a high mycorrhizal species richness (Karen and Nylund 1997). For example, despite having access to an extensive sporocarp RFLP library, Jonsson et al. (2000) were only able to match 8 taxa of the 52 total RFLP types they observed in a spruce forest in Sweden. While RFLPs of the internal transcribed spacer (ITS) are generally identical between members of the same species (Egger 1995), exceptions to this rule will result in an overestimate of species richness. An Australian study screened 109 basidiomycetes and found over one-third had multiple RFLPs within the same species, examining both ITS and mitochondrial large subunit (mtLSU) ribosomal RNA genes (Glen et al. 2001).

Bruns et al. (1998) have assembled from sporocarp specimens an extensive database of sequence information from the mtLSU ribosomal RNA gene, while other researchers have utilized the nuclear ITS region (Aanen et al. 2000), portions of the nuclear large subunit of the ribosomal RNA genes (Moncalvo et al. 2000), or both (Glen et al. 2001). These genomic regions fall upon a continuum from more conserved (i.e., mitochondrial LSU) to more variable (i.e., nuclear ITS rRNA), with conserved sequences most useful for broad taxonomic assignment (e.g., to family) while more variable regions allow finer taxonomic distinctions (e.g., species level). Recent phylogenetic analyses of fungal clades such as the agarics (Moncalvo et al. 2000), the Boletales (Grubisha et al. 2001), the Cantharellaceae (Feibelman et al. 1997), *Hebeloma* (Aanen et al. 2000) and the Inocybaceae (Matheny 2003) have dramatically expanded the sequence data available on GenBank and other global sequence databases. As a result, some groups are over-represented while others are under-represented, particularly amongst the Ascomycota.

Nevertheless, the new abundance of sequence data, combined with the plummeting cost of sequence analysis per sample, has persuaded many researchers to begin using direct sequencing of unknown fungi on roots, instead of, or in combination with, the more indirect approach of RFLP analysis (Horton and Bruns 2001).

2.3.2 Diversity of ectomycorrhizal communities

Several recent reviews have summarized the information that has emerged from these new approaches to belowground ectomycorrhizal communities from forests around the world (Taylor et al. 2000; Dahlberg 2001; Horton and Bruns 2001). Dahlberg cites over fifty community studies published since 1996, with two thirds based on morphotyping and one third using molecular methods. Since his comprehensive review was published, a number of additional studies have bolstered this number (e.g., Kernaghan and Harper 2001; Yamada and Katsuya 2001; Lilleskov et al. 2002; Zhou and Hogetsu 2002; Dickie et al. 2002b; Anderson et al. 2003; Avis et al. 2003; Landeweert et al. 2003; Nara et al. 2003; Rosling et al. 2003; Tedersoo et al. 2003).

The strongest pattern that emerges from these studies is that ectomycorrhizal communities are characterized by an impressive species richness. Horton and Bruns (2001) compared results of fourteen studies in stands composed of one or two dominant conifer species and found the number of ectomycorrhizal taxa to range from a low of seven on pine seedlings (Baar et al. 1999) to a high of 200 in 90-year-old mixed Douglas-fir/Western hemlock stands (Luoma et al. 1997), with an average of 45 taxa per site studied (Horton and Bruns 2001). Studies of belowground ectomycorrhizal communities commonly under-sample the true diversity, as revealed by species area curves (Horton and Bruns 2001; Yamada and Katsuya 2001), although lower diversity stands may be adequately sampled (Taylor et al. 2000).

The relatively high belowground diversity of mycorrhizal fungi at a given site, combined with the cost and effort required to characterize the community via morphotyping and/or molecular methods, has encouraged intensive sampling of relatively small areas (Dahlberg 1997). At this local scale, communities tend to be dominated by a few common taxa, determined primarily by the host species, but with a large number of rare taxa which are often patchily distributed (Horton and Bruns 2001). As a result, rank abundance graphs often approximate a log-normal distribution, but the curve may shift from site to site (Jonsson et al. 1999a), or by host tree or region (Taylor et al. 2000).

Bruns (1995) has proposed several possible explanations for the high levels of local-scale diversity observed in ectomycorrhizal communities. One possibility is niche differentiation through resource partitioning, which can occur both temporally and spatially. Temporal partitioning can include transitions of fungal taxa seasonally or as the host tree ages (Deacon and Donaldson 1983; Last et al. 1984; Fleming 1985; Deacon and Fleming 1992; Bigg 2000). Spatial partitioning could include taxa found in different substrate types (Goodman and Trofymow 1998a; Tedersoo et al. 2003), on different ages of roots (Gibson and Deacon 1988), or at different soil depths (Grogan et al. 2000a; Dickie et al. 2002b; Rosling et al. 2003). Small scale disturbance could also act to maintain mycorrhizal diversity at a given site. Grazing of fungal hyphae by microarthropods or other soil disturbances by soil animals could allow for continuous availability of patches for new colonization. Population-level studies of individual fungal taxa sometimes support this theory by revealing multiple small genets at a given site, i.e., for *Laccaria amethystina* (Fiore-Donno and Martin 2001), *Russula brevipes* (Bergemann and Miller 2002), *Russula cremicolor*, and *Amanita francheti* (Redecker et al. 2001). Dahlberg suggests a non-equilibrium model as an alternative possibility, in which stochasticity due to randomness of colonization maintains diversity. The fungus that happens to colonize a local site may not be a superior competitor on a larger scale, but may nevertheless succeed at that site if other fungi are distributed in a patchy manner, for example due to poor dispersal ability. These explanations probably all operate in combination, to some extent. Ectomycorrhizal fungi exist within the constraints of their interaction with host plants, the heterogeneous soil environment with its complex microhabitats and patterns of availability of nutrients and water, and dynamic interactions with other soil organisms including bacteria, decomposer and parasitic fungi, and grazing microarthropods.

Larger-scale patterns of diversity may be related to host tree distribution, variation in climate, soil characteristics, and disturbance. Molina et al. (1992) estimate that globally 5000 to 6000 species are ectomycorrhizal, however this should be considered a highly conservative estimate. This far exceeds the estimated 200 species that form arbuscular mycorrhizas, despite the much higher diversity of their associated host plants (Allen et al. 1995). Higher ectomycorrhizal diversity corresponds to a greater proportion of taxa with intermediate to high host specificity when compared to arbuscular mycorrhizas. For example, the genus *Rhizopogon*, which is specific to the Pinaceae, includes over 160 species, of which all are thought to be mycorrhizal (Molina and Trappe 1994).

Community level studies are just beginning to illuminate some of the controlling factors for regional patterns of ectomycorrhizal diversity. For example, in a study in northwestern Quebec which controlled for forest age and soil characteristics, Kernaghan et al. (2003) found that overstory tree diversity was correlated with ectomycorrhizal diversity in mixed forests of varied composition. In a study of spruce and beech forests along a north to south gradient throughout Europe, Taylor et al. (2000) found similar numbers of ectomycorrhizal taxa in spruce forests (from 14 to 19 taxa) and beech forests (from 11 to 22 taxa), but ectomycorrhizal diversity was negatively correlated with inorganic soil nitrogen levels in spruce forests, related to levels of N deposition. Further studies are needed to explore the causes and significance of global patterns of ectomycorrhizal diversity.

2.3.3 Biogeography of ectomycorrhizal communities

Due to the impressive levels of diversity revealed by community studies, it is difficult to define discrete community types or associations based on the studies performed to date. Nevertheless, some patterns have emerged when comparing forests of different regions and tree species composition.

The most extensive series of below-ground ectomycorrhizal community studies for boreal spruce/fir or pine forests have been conducted in Scandinavia (Karen and Nylund 1997; Erland et al. 1999; Mahmood et al. 1999; Jonsson et al. 1999a; Jonsson et al.

1999b; Fransson et al. 2000; Jonsson et al. 2000; Rosling et al. 2003), which can be compared with studies in Canada (Visser 1995; Kernaghan et al. 2003), northern Europe (Qian et al. 1998; Tedersoo et al. 2003), and Alaska (Lilleskov et al. 2002). Temperate forests have received less attention, with most research occurring in the western United States (Horton and Bruns 1998a; Stendell et al. 1999; Taylor and Bruns 1999) and Canada (Goodman and Trofymow 1998a; Goodman and Trofymow 1998b), but also in Japan (Yamada and Katsuya 2001) and the Netherlands (van der Heijden et al. 1999). Table 2.1 summarizes distribution of common ectomycorrhizal taxa from these studies, listing the number of sites at which a given taxon was observed, and the number of sites at which that taxon was dominant (i.e., most abundant of all EM taxa at that site). For purposes of comparison, I have omitted studies which examined only seedlings, and have included only the control plots for manipulative studies such as for effects of fertilization or litter removal.

Several species have a global distribution on a wide range of tree host species, including *Cenococcum geophilum, Thelephora terrestris,* and *Piloderma fallax*. High frequency did not always correspond to a high relative abundance. For example, *Cenococcum geophilum* was present at all sites, but only dominant at six of the 36 total sites included in Table 2.1. *Tylospora fibrillosa*, while widespread, appears from these studies to be associated with spruce forests, where it is commonly dominant or codominant (Eberhardt et al. 1999). *Cortinarius* spp., *Lactarius* spp., and *Russula* spp. were common associates of all the host trees in these studies, and were frequently dominant or codominant. This is consistent with evidence from sporocarp surveys in which these genera predominate in both temperate (Countess et al. 1998; Avis et al. 2003) and boreal (Jonsson et al. 2000) conifer forests.

Other taxa displayed some regional or host specificity but were abundant within their range. For example, *Suillus* spp. were common on scotch pine but were not found on spruces in boreal forests. *Rhizopogon* spp. were found on both Douglas-fir and bishop pine (*Pinus muricata*), but the species was different for each host and region, with *Rhizopogon vinicolor* on Vancouver Island (Goodman and Trofymow 1998a), *R. parksii*

Table 2.1 Occurrence of EMF taxa in studies of boreal and temperate forests.

Boreal Forest		**Scandinavia**		**Europe**	**Alaska**	**Canada**	
EMF taxon	*Picea abies*	Mixed forest	*Scotch pine*	*Picea abies*	*Picea glauca*	Mixed forest	**All studies**
Cenococcum geophilum	8 (8) 1*	1 (1)	8 (8) 1*	4 (4) 1*	5 (5)	2 (2) 1*	**28 (28)** **4***
Tylospora fibrillosa	8 (8) 2*	1 (1)	0 (8)	4 (4) 2*	5 (5)	0 (2)	**18 (28)** **4***
Thelephora terrestris	5 (8)	0 (1)	0 (8)	1 (4) 1*	2 (5)	0 (2)	**8 (28)** **1***
Piloderma fallax	5 (8)	1 (1)	2 (8)	1 (4)	3 (5)	1 (2)	**13 (28)** **0***
Piloderma byssinum	1 (8) 1*	1 (1)	3 (8)	0 (4)	2 (5) 1*	0 (2)	**7 (28)** **2***
Piloderma spp. total	5 (8) 1*	1 (1) 1*	5 (8)	1 (4)	3 (5) 1*	1 (2)	**16 (28)** **3***
Cortinarius spp.	6 (8) 2*	1 (1) 1*	8 (8)	3 (4)	3 (5) 1*	1 (2) 1*	**22 (28)** **5***
Lactarius spp.	5 (8)	0 (1)	3 (8)	3 (4)	5 (5) 2	0 (2)	**16 (28)** **2***
Russula spp.	3 (8) 1*	1 (1)	3 (8) 2*	4 (4)	3 (5)	1 (2)	**15 (28)** **3***
Tomentella spp.	1 (8)	1 (1)	3 (8)	2 (4)	5 (5) 1*	0 (2)	**12 (28)** **1***
Suillus spp.	0 (8)	1 (1)	8 (8) 1*	0 (4)	0 (5)	0 (2)	**9 (28)** **1***
Hebeloma spp.	0 (8)	1 (8)	5 (8)	1 (4)	2 (5)	0 (2)	**9 (28)** **0***
Wilcoxina[1]	1 (8)	1 (1)	3 (8)	2 (4)	0 (5)	0 (2)	**7 (28)** **0***
Xerocomus spp.	2 (8)	0 (8)	0 (8)	3 (4)	0 (5)	0 (2)	**5 (28)** **0***

Temperate forest	**Western US and Canada**			**Japan**	**NL**[2]	
EMF taxon	Douglas-fir	Bishop pine	***Pinus ponderosa***	***Pinus densiflora***	Creeping willow	
Cenococcum geophilum	3 (3) 2*	2 (2)	1 (1)	1 (1)	1 (1)	**8 (8)** **2***
Tomentella sublilacina	1 (3) 1*	2 (2) 1*	1 (2)			**4 (8)** **2***
Rhizopogon vinicolor	2 (3)		1 (1)			**3 (8)**
Rhizopogon parksii	1 (3)					**1 (8)**
Rhizopogon subcaerulescens		1 (2)				**1 (8)** **0***
Piloderma fallax	2 (3)					**2 (8)**
Russula spp.	1 (3)	2 (2) 1*	1 (1) 1*	1 (1) 1*		**5 (8)** **3***
Lactarius spp.	2 (3)	1 (2)	1 (1)		1 (1)	**5 (8)** **0***
Cortinarius spp.		2 (2)			1 (1)	**3 (8)** **0***

Values are numbers of studies, sites, or treatments in which the EMF taxon was present, followed in parentheses by total number of studies, sites, or treatments included. *Number of studies in which the EMF taxon was the most abundant taxon. [1]Includes EMF taxa described as E-strain or mycelium radicalis atriovirens (MRA), [2]NL=the Netherlands.

on Douglas-fir in Californian mixed forests, and *R. subcaerulescens* in pure bishop pine stands in California (Taylor and Bruns 1999). *Wilcoxina* spp. (also known as E-strain fungi) are ascomycetes in the Pezizales which appear to predominate in disturbed forest settings (Egger et al. 1991). Because *Wilcoxina* spp. tend to form a thin, inconspicuous mantle, their prevalence was initially underestimated (Egger and Fortin 1990), and they may have been missed in some of the preceding studies. Therefore, the absence of *Wilcoxina* spp. from Alaska and Canada may be an artifact of different sampling and identification procedures. *Tomentella sublilacina* was found only immediately after prescribed fire in a ponderosa pine (*Pinus ponderosa*) forest (Stendell et al. 1999), but was common on white spruce (*Picea abies*) at all 5 sites in Alaska (Lilleskov et al. 2002), and was dominant in California on Douglas-fir in mixed stands (Horton and Bruns 1998a) and on bishop pine (Taylor and Bruns 1999). While *Tomentella sublilacina* rapidly colonized seedlings from resistant soil propagules after a wildfire, it was unique in also being associated with mature bishop pine roots before the fire (Baar et al. 1999).

Thelephoroid resupinates such as *Tomentella* spp. and *Pseudotomentella tristis* have only recently been recognized as important ectomycorrhizal fungi (Koljalg et al. 2000), and may not have been identified in some of the preceding studies. Several of these studies had a high proportion of unidentified taxa which complicates direct comparison of the findings. For example, Goodman and Trofymow (1998a; 1998b) identified only five taxa of the 69 morphological types observed. Similarly, Yamada and Katsuya (2001) identified only seven of the 54 morphological types observed. In molecular studies, some taxa may be overlooked due to problems with universality of primers. Variation in sampling effort may also result in disparities between observations of rare taxa, even where the majority of samples are successfully identified (Horton and Bruns 2001).

2.4 Ecosystem controls and disturbance impacts on ectomycorrhizal communities

As knowledge of the composition of ectomycorrhizal communities has expanded, attention has increasingly been focused on investigating impacts of natural or human disturbances. Studies have evaluated the importance of substrate type (e.g., coarse woody debris, buried soil wood, forest floor, mineral soil) (Harvey et al. 1978; Harvey et al. 1979; Kropp 1982; Goodman and Trofymow 1998a; Tedersoo et al. 2003), soil depth (Dickie et al. 2002b; Rosling et al. 2003), and soil chemistry (Qian et al. 1998; van der Heijden et al. 1999; Kernaghan et al. 2003). Early reports of reductions in ectomycorrhizal sporocarp production (Arnolds 1991) have stimulated studies on the potential impacts of fertilization (Termorshuizen 1993; Nilsen et al. 1998; Fransson et al. 2000; Jonsson et al. 2000; Avis et al. 2003) and high rates of nitrogen deposition due to industrial pollution (Karen and Nylund 1997; Erland et al. 1999; Taylor et al. 2000; Lilleskov et al. 2002). Another area of concern has been the impact of wildfire (Miller et al. 1998; Horton et al. 1998b; Baar et al. 1999; Visser and Parkinson 1999; Grogan et al. 2000a; Jones et al. 2003) and prescribed fire (Herr et al. 1994; Stendell et al. 1999) on soil inoculum potential and the resultant ectomycorrhizal community on seedlings regenerating after fire. Finally, a number of studies have addressed consequences for mycorrhizal communities of widespread forest harvesting (Kranabetter and Wylie 1998; Kranabetter et al. 1999; Hagerman et al. 1999a; Byrd et al. 2000).

2.4.1 Substrate type, soil depth, and soil chemistry

Natural forest soils are rarely a homogeneous medium, and evidence is accumulating that the fine scale distribution of many ectomycorrhizal fungi is patterned by patches of soil substrate such as buried wood. Whether or not this can be extended to greater spatial scales to explain ecosystem-level attributes of ectomycorrhizal diversity and community composition remains to be established. Certain taxa may be specialized for certain substrates such that they are essentially excluded from ecosystems where those substrates are not available. For example, *Piloderma fallax* appears to be associated with highly decayed coarse woody debris. In Douglas-fir forests in Oregon, *P. fallax* was two and a half times more likely to occur in plots with coarse woody debris in decay class 5 and its chance of occurrence was closely related to the amount of decayed wood present (Smith et al. 2000). Goodman and Trofymow (1998a) found spatial patterning by substrate in old-growth and mature Douglas-fir forests on Vancouver Island, with *Piloderma fallax* in woody substrates, two unidentified morphological types confined to the forest floor, and one found only in mineral soil. Conn and Dighton (2000) observed differences in dominant ectomycorrhizal morphotypes growing in pine litter compared to oak litter. In molecular studies, where a greater majority of taxa can be identified, substrate patterns are more distinct. In an Estonian mixed forest, Tedersoo et al. (2003) used detrended correspondence analysis (DCA) to reveal clear separation of ectomycorrhizal communities in coarse woody debris with respect to mineral soil (A and E horizons). Once again *Piloderma* was associated with coarse woody debris, along with *Tomentella*, *Amphinema*, and *Tylospora*.

Ectomycorrhizal communities also appear to be patterned to some extent based on soil horizon, particularly with respect to organic horizons compared to mineral soil. Rosling et al. (2003) found *Piloderma fallax* only in upper horizons, but three other unidentified *Piloderma* spp. were found only in the deeper B and C horizons. Similarly, *Russula decolorans* was abundant in the organic and E horizon, but it was replaced by *Russula adusta* in deeper horizons. It is interesting to note that these patterns would have been overlooked in the absence of species-level identification. Of the 22 total taxa identified, two were confined to the organic horizon, 11 were found only in mineral horizons, and three were confined to the deeper C horizon. Spatial patterns of root tips can only approximate the position of the fungal mycelium for each taxon. In an innovative approach to this issue, Dickie et al. (2002b) used molecular methods to identify fungal hyphae in the soil of a 60-year-old red pine stand in Pennsylvania to distinguish groups of taxa associated with different soil layers. Approximately half the fungal taxa were broadly distributed, while nine taxa including a *Lactarius* species were mostly confined to the litter layer, and several including *Amanita vaginata* predominated in the mineral layer. Changes in substrate availability may not always impact ectomycorrhizal communities, however. Experimental removal of forest residues (i.e., slash) over a 30-year period from a white spruce forest in Sweden reduced the thickness of the litter layer and reduced total mycorrhizal colonization of roots, but had no significant effect on the ectomycorrhizal community composition compared to control plots (Mahmood et al. 1999).

The relative significance of soil chemistry of natural systems in controlling the distribution and abundance of ectomycorrhizal fungi is not yet clear. While studies sometimes reveal shifts in abundance among individual taxa, the effect on overall species richness and species composition may be minimal. Kerneghan et al. (2003) found that increased mycorrhizal diversity was more closely correlated to overstory tree diversity than changes in soil chemistry, although abundance of *Russula* and *Cenococcum*, the two codominants, were positively correlated with increases in soil exchangeable cations. Soil chemistry is strongly influenced by litter input, and therefore it is difficult to distinguish direct effects of soil chemistry from changes due to host specificity. In a simpler system with creeping willow as the only host, van der Heijden et al. (1999) used canonical correspondence analysis (CCA) to conclude that autumn ammonium and summer pH levels were significant in explaining the composition of ectomycorrhizal morphotypes. Experimental manipulation of soil chemistry through six years of liming and acidification of a white spruce stand in Bavaria (Qian et al. 1998) resulted in shifts in the belowground ectomycorrhizal community. While the treatments were unreplicated and therefore statistical analysis could not be performed, reduced pH appeared to favor *Xerocomus badius* over *Piceirhiza nigra* (probably a tomentelloid species) and *Tuber puberulum*, while liming reduced the abundance of the dominant taxon, *Russula ochroleuca* in favor of *Piceirhiza nigra*.

2.4.2 Community effects of fertilization and nitrogen deposition

Alarming declines in fruiting of ectomycorrhizal taxa in parts of Europe subjected to high levels of industrial nitrogen pollution have motivated a series of studies of above and belowground responses of ectomycorrhizal fungi to nitrogen (Arnolds 1991). Exploratory studies of forest stands located along natural gradients of nitrogen deposition levels have generally revealed reductions in species richness and characteristic shifts in abundance of some ectomycorrhizal taxa in areas with greater N pollution (Erland et al. 1999; Taylor et al. 2000; Lilleskov et al. 2002). In southern Swedish white spruce forests, the decrease in species richness at the site with high N deposition was mostly due to the absence of *Cortinarius* spp. (Erland et al. 1999). Absence of *Cortinarius* spp. was also partly responsible for lower species richness, while dominance of *Tylospora* sp. and *Lactarius* spp. reduced evenness in a white spruce forest in Germany compared to other sites with lower N deposition in France and Scandinavia (Taylor et al. 2000). The strongest evidence of impacts of nitrogen deposition comes from a study of white spruce (*Picea glauca*) forests down wind from a fertilizer facility in Alaska, in which ectomycorrhizal species richness declined from 30 taxa to nine along a gradient of increasing N deposition. Again, *Cortinarius* spp. were absent from high N sites, while *Lactarius theiogalus* became the new dominant taxon. *Cenococcum geophilum* was present at low abundance at all sites, while *Tomentella* spp. showed a species-specific pattern, with *Tomentella sublilacina* most abundant with moderate to high N levels, and *Tomentella stuposa* and other unidentified *Tomentella* spp. found only at low N levels.

Fertilization studies have revealed a more complex picture of effects of N addition. White spruce seedlings grown in pots with added ammonium nitrate showed no significant difference in colonization levels or abundance of the four morphological types observed (Nilsen et al. 1998). Likewise, in young stands of scotch pine in the Netherlands fertilized over a two year period, Termorshuizen (1993) found that reductions in fruiting did not necessarily correspond to reductions in total abundance of ectomycorrhizal fungi on roots, although he did not identify ectomycorrhizal taxa. Longer-term fertilization studies have since confirmed that nitrogen addition reduces aboveground fruiting while belowground colonization rates remain stable (Karen and Nylund 1997; Jonsson et al. 2000; Avis et al. 2003).

Patterns of species richness and abundance of individual taxa have proved to be more revealing than colonization rates in some studies. In a Swedish white spruce forest fertilized for one to four years, ectomycorrhizal fungi identified by RFLP analysis failed to exhibit any changes in species richness, and there were no consistent shifts in abundance of individual taxa including the dominant *Cenococcum geophilum*, although patterns may have been obscured by high annual variability (Jonsson et al. 2000). In contrast, field studies have found evidence of significant shifts in mycorrhizal communities under the influence of chronic fertilization when evaluated over longer time periods. In 36-year-old stands of white spruce in Sweden fertilized with ammonium nitrate over a ten-year period, principal components analysis (PCA) revealed a unique ectomycorrhizal assemblage, identified by RFLP analysis, when compared to control stands or irrigation alone (Fransson et al. 2000). While there were no changes in total colonization rates or total species richness, *Cenococcum geophilum* abundance increased significantly in fertilized plots. Similarly, oak savannas subjected to 16 years of ammonium nitrate fertilization revealed significant shifts in ectomycorrhizal communities with dominance of *Cortinarius* spp. in unfertilized plots and of *Russula* spp. in fertilized plots (Avis et al. 2003). Unlike Fransson et al. (2000), Avis et al. (2003) found *Cenococcum geophilum* abundance to be unaffected by fertilization, while total species richness and evenness was noticeably reduced in fertilized plots.

2.4.3 Community effects of wildfire and prescribed fire

Stand replacing fires may influence mycorrhizal communities by killing roots and mycelium in organic soil horizons and thereby impacting inoculation potential, by changing availability of soil nutrients and water, and by altering the species composition and age structure of the forest. It may be difficult to separate these factors in evaluating the impacts of fire. Prescribed fires generally kill smaller trees and understory plants, which changes the age structure of the forest and may result in elimination of shade-tolerant tree species. Nevertheless, dominant trees remain as hosts for mycorrhizal fungi, allowing comparison of ectomycorrhizal communities before and after the fire without the complication of comparing mature tree to seedling communities. While prescribed fires are generally lower intensity than wildfires, organic horizons are still reduced or eliminated. In a native ponderosa pine forest in California, changes in belowground ectomycorrhizal biomass were related to loss of the litter layer, while biomass of mycorrhizas in the mineral soil horizons was not affected by prescribed fire. *Tomentella sublilacina* and a *Martellia* species (Russulaceae) dominated in fire plots, while another Russuloid species and a Thelephoroid species dominated control plots, with *Rhizopogon subcaerulescens* common to both fire and control plots (Stendell et al. 1999).

Several studies have examined colonization of seedlings to assess the inoculation potential of soils after fires of varying intensity. Effects of fire on inoculation potential have been evaluated by sampling seedlings naturally regenerated after wildfire (Miller et al. 1998; Horton et al. 1998b), by planting seedlings into burned sites (Herr et al. 1994), and by greenhouse studies in which seedlings are inoculated with soil from burned sites (Baar et al. 1999). In general, these studies have identified a small subset of ectomycorrhizal taxa which predominate immediately after fire. Sampling of bishop pine seedlings naturally regenerating one year after a wildfire in Point Reyes, California revealed dominance of *Rhizopogon* species, *Wilcoxina mikolae*, and *Tomentella sublilacina* (Baar et al. 1999). These taxa were shown to colonize seedlings in bioassays with soil from the burned site, even after experimental drying for one month. Resistant propagules apparently were able to persist for long periods of time, since both *Rhizopogon* and *Wilcoxina mikolae* were rare in the mature forest before the wildfire (Baar et al. 1999). In a study of the same fire, in burned areas formerly dominated by chaparral scrub plants associated with vesicular arbuscular mycorrhizal fungi, seedlings had only *Rhizopogon subcaerulescens, R. ochraceorubens*, and *Suillus pungens*. Seedlings growing in the adjacent burned forest had ten additional ectomycorrhizal taxa including *Russula brevipes* and *Tuber californicum*, some of which may have been dependent on direct colonization by surviving hyphal fragments. Colonization of seedlings by *Rhizopogon* and *Suillus* in the VAM-dominated areas demonstrated that these taxa establish readily from spores which are both fire resistant and effectively dispersed (Horton et al. 1998b). Further intensive sampling of seedlings at Point Reyes revealed a pattern of colonization consistent with randomly distributed (i.e., not clumped) sources of inoculum in the upper mineral soil, suggesting that many seedlings acquire their ectomycorrhizal fungi predominantly from spores, at least initially after fires (Grogan et al. 2000a).

Shifts in ectomycorrhizal communities could also be due to alteration of the physical or chemical environment of the soil after fire. Burning of organic residues creates ash, often resulting in increases in pH and mobilization of inorganic nitrogen (Grogan et al. 2000b). Nevertheless, an experimental investigation of the effects of ash removal over a one and a half year period at Point Reyes found no significant effect on ectomycorrhizal diversity or community composition compared to burned sites where ash was allowed to remain (Grogan et al. 2000a).

The preceding studies addressed short-term impacts of fire, but ultimately it is important to explore the longer-term impacts of wildfire on ectomycorrhizal communities. In scotch pine stands in northern Sweden, Jonsson et al. (1999b) devised an approach to this question by comparing unburned stands to adjacent stands which had experienced low intensity wildfires one to 62 years previously. Of the 135 mycorrhizal taxa identified based on RFLP analysis, most of the common fungal taxa did not appear to be affected by prior fire history, which also had no effect on species richness. While previously burned stands had a lower evenness due to strong dominance by a single taxon at each site, the dominant taxon differed from site to site, with an ascomycete at the 1-year fire site, *Suillus variegatus* at the 13 year fire site, and an unknown taxon and *Cenococcum geophilum*, respectively, at the two 62 year fire sites. The authors concluded that spatial variation outweighed the effects of low-severity fire in these boreal forests (Jonsson et al. 1999b).

2.4.4 Effects of forest harvesting on ectomycorrhizal communities

Like wildfire, forest harvesting changes the age distribution and the species composition of forest overstory and understory, and alters physical, chemical, and biological components of the soil environment which ectomycorrhizal fungi inhabit. Clearcut logging removes all overstory trees, although non-commercial tree species and seedlings ("advance regeneration") may persist, and it is important to note that root systems may remain viable more than two years after harvesting (Harvey et al. 1980; Hagerman et al. 1999a), which may help to maintain local fungi until their host trees are reestablished at the site. Site preparations before planting, such as broadcast burning, soil treatments like scarification, or understory plant removal may increase the impacts on ectomycorrhizal communities (Amaranthus 1990). Thinning operations, while not removing all mature trees, may result in soil disturbance similar to that incurred by clearcutting, while removal of trees and death of their associated fungi could create gaps in which new fungal taxa might establish.

A four-year study of effects of thinning on 14-year-old scotch pine stands in Britain showed relatively minor shifts in ectomycorrhizal sporocarp production, although belowground abundance was not assessed (Shaw et al. 2003). Hypogeous sporocarp production was also not significantly different in thinned and unthinned 70-year-old white fir (*Abies concolor*) stands in California measured ten and seventeen years after thinning, but again, significant shifts were observed in ectomycorrhizal species composition (Waters et al. 1994). Belowground ectomycorrhizal communities responded to thinning with significant decreases in abundance and species richness in late-successional Douglas-fir forests in Oregon (Stockdale 2000). For the stand as a whole, 61 morphotypes were identified before thinning, while 47 types were identified after thinning, a 23% decline. While soil cores outside the dripline showed a significant decrease in morphotype species richness, cores within the dripline of residual trees had no significant loss of species richness due to thinning. The most common taxa were unaffected by thinning, and thus the stand level reduction in species richness apparently was caused by loss of rare taxa, although this could have been a consequence of reduced root density (smaller effective sample size) instead of a direct treatment effect (Stockdale 2000).

Impacts of clearcutting on ectomycorrhizal communities could result from a number of causes: loss of inoculation potential, decreases in carbon input from host plants, or changes to the soil environment. Some studies have shown decreases in colonization of seedlings planted in clearcuts, especially in harsh sites where regeneration may be unsuccessful (Perry et al. 1987). Nevertheless, studies of soil inoculation potential generally reveal sufficient quantities of resistant propagules to allow seedlings to become fully colonized (e.g. Pilz and Perry 1984), suggesting that other factors may be responsible in cases where colonization of seedlings in clearcuts is limited. In reviewing 15 studies of ectomycorrhizal fungi on seedlings after clearcutting, Jones et al. (2003) found little support for the idea that loss of inoculation potential in clearcut soil limits colonization, since bioassays of clearcut soils and field experiments in clearcuts showed no consistent effect on degree of colonization. Instead, they proposed that changes in soil biology and chemistry after harvesting select for fungi better suited for the altered environment, resulting in a shift in ectomycorrhizal communities on seedlings regenerating in clearcuts as opposed to forests (Jones et al. 2003).

Recently, several intensive assessments of seedling ectomycorrhizal communities have been completed, revealing a substantial species richness on seedlings in clearcuts, using either morphotyping (Roth and Berch 1992; Kranabetter and Wylie 1998; Kranabetter et al. 1999; Hagerman et al. 1999a) or RFLP analysis (Hagerman et al. 1999b; Byrd et al. 2000), although seedlings in non-native plantations may prove an exception. For example, Sitka spruce (*Picea sitchensis*) seedlings in 2 ha forest gaps in an uneven-aged plantation forest in Scotland had only 13 morphotypes with overwhelming dominance by *Tylospora fibrillosa* (Flynn et al. 1998). Roth and Berch (1992) found 33 morphotypes on Douglas-fir seedlings one season after outplanting in clearcuts on Vancouver Island, including the dominant *Thelephora terrestris*, with lesser abundance of *Rhizopogon vinicolor* and *Cenococcum geophilum*. Kranabetter et al. (1999) planted lodgepole pine, white spruce, and subalpine fir seedlings near forest edges of clearcuts in Canada, identifying an average of 52 morphotypes per seedling species with a total of 74 morphotypes. Nearly 60% of the taxa were found on more than one of the three seedling species, including the dominant taxa *Cenococcum geophilum*, MRA (probably a *Wilcoxina* species), and *Amphinema byssoides* (Kranabetter et al. 1999). A study of *Pinus contorta* saplings in eight-year-old clearcuts near Yellowstone National Park found slightly lower species richness of 50 taxa compared to 66 taxa in adjacent undisturbed forests using RFLP analysis. Although only ten of the total 106 taxa identified were shared between clearcut and undisturbed forest, shared taxa accounted for nine of the fourteen most common taxa. Two species in the Russulaceae and one species in the Thelephoraceae were common in the clearcut, while a species in the Tricholomataceae was common in the undisturbed forest, and *Cenococcum geophilum* and several Suilloid species were common in both clearcut and forest. Community distance measures revealed that differences between clearcut and forest exceeded site differences (Byrd et al. 2000).

At the landscape level, clearcutting often differs from natural disturbance regimes in both extent and severity, which might impact mycorrhizal diversity by changing the likelihood that regenerating seedlings would interact with root systems of surviving mature trees. Studies of seedlings in various size clearcuts or growing near forest edges have helped to evaluate the relative significance of intact mycelial connections with mature trees, in ectomycorrhizal colonization of seedlings. Hagerman et al. (1999a) measured the persistence of ectomycorrhizas in soil cores two and three growing seasons after clearcutting of a subalpine fir/Engelmann spruce (*Picea engelmannii*) forest in Canada, to compare with ectomycorrhizas on spruce seedlings planted at varying distances from the forest edge (Hagerman et al. 1999b). After the first growing season, as the roots of harvested trees began to die, the abundance and diversity of ectomycorrhizas in soil cores decreased with distance from the forest edge. By year three, species richness decreased from over 7 morphotypes per core in the forest to less than 2 morphotypes per core for samples at least 16 m from the forest edge (Hagerman et al. 1999a). Ectomycorrhizas on seedlings followed a similar although less extreme pattern, with significant decreases in species richness from over 5 morphotypes per seedling planted 2 m from the forest edge compared with less than 4 morphotypes per seedlings at least 16 m into the clearcut (Hagerman et al. 1999b). Most of the common ectomycorrhizal taxa in cores were also found on seedlings, with the exception of two *Lactarius* species.

Several other Canadian studies have reported similar decreases in mycorrhizal fungal species richness of seedlings with increased distance into clearcuts. For western hemlock and lodgepole pine seedlings two years after planting, ectomycorrhizal fungal species richness per seedling decreased beyond 7 m into clearcuts from intact forest edges (Durall et al. 1998). For naturally regenerated western hemlock seedlings in four-year-old clearcuts at varying distance from forest edges, Kranabetter and Wylie (1998) reported decreases in morphotypes per seedling from 13.1 in the forest to 9.6 at the forest edge to 7.8 in the clearcut. The majority of fungal taxa were found on seedlings regardless of position, but nine taxa, including a *Russula* species, a *Tuber* species, and *Piloderma croceum* (= *P. fallax*) were found in the forest and at the forest edge but not in the clearcut. A follow-up study in which mycorrhizal forest seedlings were transplanted into forest openings suggested that decreases in fungal species richness in the opening were caused by inability of mature forest fungi to persist in that environment (Kranabetter and Friesen 2002). It was not clear whether changes in the soil environment or loss of hyphal linkages was responsible for the exclusion of mature forest fungi.

While few studies have examined the influence of isolated trees on mycorrhizal fungal diversity of seedlings, effects appear to be similar to those observed for seedlings growing near forest edges. Kranabetter (1999) found that paper birch seedlings had higher mycorrhizal fungal diversity when growing near paper birch trees in clearcuts and in mixed conifer forest. It appeared that the effect of proximity to conspecific trees outweighed the impacts of harvesting. For seedlings near paper birch trees, mycorrhizal fungal diversity was similar for seedlings in clearcuts and in forests. Dickie (2002a) found that red oak (*Quercus rubra*) seedlings growing near living (stump-sprouting) red oak stumps had higher mycorrhizal colonization rates and mycorrhizal diversity than seedlings growing near dead stumps or live red maple (*Acer rubrum*) stumps, which are arbuscular mycorrhizal.

Hyphal linkages to mature trees have been proposed to provide an essential extra source of energy for colonization of seedlings by mycorrhizal fungi taxa with high energy demands (e.g., Deacon and Donaldson 1983; Fox 1986). Severing of hyphal linkages to trees resulted in the absence of a *Lactarius* species from seedlings, while other mycorrhizal fungal taxa were unaffected (Fleming 1983; Fleming 1984). Soil trenching of Douglas-fir seedlings in 90- to 120-year-old Douglas-fir/paper birch forests in British Columbia resulted in decreases in species richness from 17 taxa on untrenched seedlings to nine taxa on trenched seedlings, with a marked decrease in abundance of taxa forming rhizomorphs (Simard et al. 1997d). MRA-type fungi were dominant on both trenched and untrenched seedlings, but *Rhizopogon vinicolor* increased 20-fold on untrenched seedlings, while *Thelephora terrestris* was more abundant on trenched seedlings. *Lactarius deliciosus*, a *Tuber* species, and an *Amphinema* species were found only on the untrenched seedlings.

2.5 Successional changes in ectomycorrhizal communities

An abundant record of studies documents successional progression in ectomycorrhizal sporocarp production (Mason et al. 1983; Last et al. 1984; Last et al. 1987; Deacon and Fleming 1992; Countess et al. 1998; Jumpponen et al. 1999; Bigg 2000; Smith et al. 2002; Nara et al. 2003; Shaw et al. 2003). Successional progression of ectomycorrhizal fungi producing sporocarps in association with birch trees in Scotland (Last et al. 1984), associated with inability of late fruiting taxa to colonize seedlings in the absence of mycelial connections to mature tree roots (Fleming 1983), led to the classification of fungal taxa as either early-stage or late-stage (Deacon and Donaldson 1983; Fox 1983; Mason et al. 1983; Fox 1986). Early stage fungi were proposed to adhere to a ruderal strategy, colonizing roots readily from spores, while late stage fungi had greater photosynthate requirements and therefore were dependent on mycelial connections to mature trees. It should be noted that these studies were carried out in agricultural fields with birch trees planted from non-native seed sources, therefore the ectomycorrhizal community would be expected to be depauperate, biased towards non-host-specific taxa, and predominantly established through long-distance spore dispersal.

The disparity between above and belowground measurement of ectomycorrhizas has inspired renewed interest in assessing successional changes of belowground communities in native forests. Studies in native forests have revealed a more complex pattern than that portrayed by succession of sporocarps. Information about succession may be derived from mapping or tracking persistence of genets in population studies (Villeneuve et al. 1991; Selosse et al. 1998; Redecker et al. 2001; Sawyer et al. 2001; Bergemann and Miller 2002; Guidot et al. 2004), comparing seedling mycorrhizas to roots of mature trees (Jonsson et al. 1999a), or via a chronosequence, by comparing forests of different ages (Visser 1995; Goodman and Trofymow 1998b; Byrd et al. 2000).

Population studies paint a complex picture, revealing a surprising persistence of some genets, while most genets appear to be small and subject to frequent turnover. An inoculated strain of *Laccaria bicolor* was persistent on Douglas-fir seedlings ten years after outplanting. Furthermore, the introduced strain did not replace native *Laccaria bicolor* genets in adjacent uninoculated plots, leading the investigators to conclude that distribution of *Laccaria bicolor* was a product of mycelial outgrowth and competition with conspecifics rather than establishment of new genets via spore dispersal (Selosse et al. 1998). In a *Pinus radiata* plantation in Australia, *Amanita muscaria* from several widely separated sites were from the same genetic individuals, implying that genets introduced at planting had persisted up to 36 years (Sawyer et al. 2001).

In contrast, studies in mature forests have revealed that even for supposed late-stage fungi, genet sizes are often quite small. In mature bishop pine forests, Redecker et al. (2001) found maximum areas for *Russula cremoricolor* genets of 1.1 m^2, for *Amanita francheti* 1.5 m^2, and for *Lactarius xanthogalactus* 9.3 m^2. Bergemann and Miller (2002) encountered similar genet sizes for *Russula brevipes* in mature lodgepole pine and Sitka spruce stands. It is notable that *R. brevipes* genets in the spruce stands were closely related, suggesting that sexual recombination was common, while genets in the pine stands were unrelated, implying that the population had established from multiple sources, i.e., colonization from long-distance spores. Kretzer et al. (2004) determined that genet size can differ substantially between species of the same genus: *Rhizopogon vesiculosus* spanned up to 13.4 m while that largest *R. vinicolor* genet was only 2 m across. In a ten year sporocarp mapping study, Guidot et al. (2004) found evidence of rapid turnover of *Hebeloma cylindrosporum* mycelium under sporocarps: DNA was detectable in soil directly under each sporocarp, but absent one year later in the same site.

Ideally, successional changes would be traced from stand establishment through maturity, but in the absence of long-term records of belowground ectomycorrhizal communities, chronosequences provide a critical source of information. Many chronosequence studies have discovered relatively strong similarities between ectomycorrhizal communities in forests of different successional ages, consistent with persistence of taxa establishing early, but with addition of a few taxa in later successional stages. In *Pinus banksiana* stands regenerating after wildfire, Visser (1995) distinguished early-stage, multi-stage, and late-stage taxa but observed that few taxa present in 6-year-old stands were completely replaced in the older stands. Instead, over the first 40 years after fire, addition of late stage taxa including *Cortinarius* spp., *Lactarius* spp., *Russula* spp., *Tricholoma* spp., and *Piloderma byssinum* shifted the species abundance distribution from a geometric series dominated by a few taxa to a more even community approaching a lognormal distribution. Likewise, the ectomycorrhizal community in undisturbed *Lodgepole pine* stands was significantly different than in eight-year-old clearcuts, but 9 of the 14 most abundant taxa were common to both treatments (Byrd et al. 2000). Using morphotyping, Goodman and Trofymow (1998b) found no significant differences between ectomycorrhizal communities from mature (87- to 89-year-old) and old-growth (288- to 441-year-old) Douglas-fir stands. Mature stands had 55 distinct morphotypes while old growth stands had 53 morphotypes, and the six most abundant taxa were common to both forest types.

Chapter 3. Spatial Effects of Retention Trees on Growth and Mycorrhizal Attributes of Douglas-fir Seedlings

3.1 Introduction

Management strategies involving retention forestry have been proposed as an alternative to clear-cutting of Douglas-fir forests in the Pacific Northwest (Kohm and Franklin 1997). While patterns and densities of residual trees can vary, retention forestry (or "green-tree retention") refers to harvesting in which some proportion of the living trees are protected. Residual trees can lessen the impacts of harvesting by reducing erosion, moderating the effects of wind and solar exposure, and harboring organisms that would otherwise be excluded by clear-cutting (Perry et al. 1989a; North et al. 1996). Mounting concern over the impacts of clearcutting has led to widespread adoption of provisions for retention of green trees, snags, and logs by private (WFPA 1995) and public (FEMAT 1993; WFPB 1995) forest managers throughout the Pacific Northwest. However, more testing is needed to determine the impacts and benefits of retention forestry.

In the western foothills of the Cascade mountain range, Douglas-fir is considered to be shade-intolerant and unable to regenerate under a closed canopy (Franklin and Dyrness 1988). Survival and growth of Douglas-fir seedlings after partial retention harvesting may depend on the density of residual trees. In mature Douglas-fir stands in western Oregon thinned to a basal area of 16-31 m^2/ha, planted Douglas-fir seedlings had low survival rates (Brandeis et al. 2001). However, Edmonds et al. (2000) found no significant difference in Douglas-fir seedling survival over the first growing season in seedlings planted from 1 m to 6 m from retention trees in western Washington. While seedlings near trees would be expected to experience increased competition, residual trees might also facilitate seedling survival and growth. Root systems could enhance seedling colonization by mycorrhizal fungi, helping the regenerating stand to retain a functionally diverse array of mycorrhizal fungi.

A number of studies have documented differences in the mycorrhizal status of seedlings planted within the rooting zone of residual trees or of forest edges. Dickie et al. (2002a) demonstrated that oak seedlings near harvested, stump-sprouting mature oak trees had greater mycorrhizal colonization and diversity, which they attributed to a "nurse tree" effect. In northwest British Columbia, paper birch seedlings had higher mycorrhizal diversity when growing near mature paper birch trees both in clearcuts and in mixed conifer forests (Kranabetter and Wylie 1998). In the same region, planted western hemlock and lodgepole pine seedlings (Durall et al. 1998) and naturally regenerated western hemlock seedlings (Kranabetter and Wylie 1998) near the mature forest edge had higher EMF diversity than seedlings farther in from the forest edge. When naturally regenerated western hemlock seedlings from the forest were transplanted into clearcuts, mycorrhizal diversity declined (Kranabetter and Friesen 2002). In clearcuts in the southern Interior of British Columbia, Hagerman et al. (1999b) showed that hybrid Engelmann spruce x white spruce bioassay seedlings planted 2 m from forest edges had higher EMF species richness than seedlings planted 16 m into clearcuts, although proximity to the forest edge had no effect on previously colonized Engelmann spruce seedlings planted in mineral soil exposed by mechanical mounding (Jones et al. 2002). EMF species richness of planted western hemlock and lodgepole pine seedlings decreased beyond 7 m distance from the forest edge into clearcuts (Durall et al. 1998). These studies provide support for the theory that residual trees could enhance mycorrhizal colonization of seedlings, although Jones et al. (2003) point out that shifts in the EMF community in clearcuts could be adaptive, in response to changes in the soil chemistry, biology, or other aspects of the physical environment.

The effect of proximity to residual trees on Douglas-fir seedling growth and mycorrhizal status has not been well investigated in the Pacific Northwest, although retention forestry has recently been widely adopted in management of western Douglas-fir forests. Because maintenance of biodiversity has increasingly become a priority in management of both public and private forest lands, it is necessary to evaluate the effectiveness of partial canopy retention for promoting diversity of EMF communities in regenerating stands.

The objective of this study was to assess the spatial influence of residual trees on growth and mycorrhizal attributes of Douglas-fir seedlings. The following hypotheses were tested: (i) seedlings near residual trees have higher levels of mycorrhizal colonization, (ii) seedlings near residual trees have higher levels of mycorrhizal diversity per seedling than a) seedlings growing beyond the root zone of residual trees and b) seedlings exposed to soil propagules but deprived of root connections, (iii) seedlings growing near residual trees experience growth inhibition due to shading or other competitive interactions, and (iv) seedling growth rates are influenced by mycorrhizal status.

3.2 Materials and methods

3.2.1 Study sites and characteristics

The three study sites were located in the western foothills of the Cascade mountain range in Washington State, approximately 50 km southwest of the city of Seattle. The sites were between 7 and 13 km in distance from each other. Two sites, “Beatles” (47° 20.970’ N, 121° 49.895’ W) and “Imagine” (47° 23.956’ N, 121° 48.938’ W), were in the Cedar River watershed, managed by the City of Seattle, while one site, “Green River” (47° 18.719’N, 121° 42.609’ W) was located in the adjacent Green River watershed, on land owned and managed by the Plum Creek Timber Company. The three sites represented a chronosequence of time since harvest, with the Green River site harvested only two years before the study began, while Imagine was harvested four years and Beatles six years before the study commenced in 1998.

The Green River site was different from the other two sites in several important respects. The forest was younger at the time of harvest at the Green River site, reflected by the age of the center tree of 44 years versus 72 and 69 years at Imagine and Beatles, respectively (Table 3.1). The center trees at Imagine and Beatles were consequently both

Table 3.1 Site harvest dates, elevation, soil characteristics, and Douglas-fir center tree attributes.

	Site		
	Green River	Imagine	Beatles
Year of harvest	1996	1994	1992
Elevation (m)	808	457	488
Soil pH	4.22 ± 0.075*a*	3.87 ± 0.045*b*	3.99 ± 0.066*b*
Soil moisture (%)	76 ± 6.6	91 ± 8.4	92 ± 8.1
Center tree attributes			
Age (y)	44	72	69
Height (m)	34	44	42
DBH (cm)	43.4	66.3	62.5

Soil pH and moisture are means ± 1 SE averaged from monthly growing season soil cores, with 4 replicates per sample date and a total of 36 cores for Green River and 40 cores for Beatles and Imagine, respectively. Soil moisture was calculated as percent dry weight. Within rows, means with the same letter are not significantly different by Tukey's honestly significant difference test (α=0.05).

taller and larger in diameter than the Green River center tree, but were similar in age and size to each other. Averaged monthly over the growing season, the soil pH from 20 cm deep soil cores was relatively acidic at all three sites, but was less acidic at Green River (4.2) than at Imagine (3.9) and Beatles (4.0). Soils at Green River were drier, on average, than at the other sites (Table 3.1), but the differences were not significant when compared by month, by year, or over the entire duration of the study (data not shown). Due to its higher elevation, Green River would be expected to receive greater amounts of precipitation than the other sites. While the other two sites were flat, the Green River site had a 10° slope and faced southwest. This southwestern exposure could have compensated for any differences in precipitation, resulting in drier soils, especially during the summer months.

Vegetation at the sites was typical of low elevation forest clearcuts and early successional forests. In addition to residual Douglas-fir trees and planted seedlings, other conifer species at the sites included young and mature western hemlock, Pacific silver fir (*Abies amabilis* (Dougl.) Forbes), western red-cedar (*Thuja plicata* (L.) Donn.), and grand fir (*Abies grandis* (Dougl.) Forbes).

The sites shared many of the same understory plants with a few species found at only one or two sites (Table 3.2). Red huckleberry (*Vaccinium parvifolium* (L.) Smith), thimbleberry (*Rubus parviflorus* (L.) Nutt.), salmonberry (*Rubus spectabilis* (L.) Pursh), Pacific blackberry (*Rubus ursinus* (L.) Cham. & Schlecht.), sword-fern (*Polystichum munitum* (Kaulf.) Presl.) and bracken fern (*Pteridium aquilinum* (L.) Kuhn.) were common at all three sites. While vine maple (*Acer circinatum* (L.) Pursh) and red alder (*Alnus rubra* (Hill) Bong.) occurred within the sampling area at both Green River and Imagine, these species were observed near but not within the sampling area at Beatles. The Beatles site featured thickets of Himalayan blackberry (*Rubus discolor* (L.) Weihe & Nees*)* and evergreen blackberry (*Rubus laciniatus* (L.) Willd.). Imagine differed from the other sites in the presence of twinflower (*Linnea borealis* var. *longiflora* (L.) Torr.) and Oregon grape (*Berberis nervosa* (L.) Pursh).

The understory vegetation at Imagine was dominated by salal (*Gaultheria shallon* (L.) Pursh), which approached 100% coverage in parts of the sampling area. Notably, salal was common at Beatles as well, but was not observed at Green River. This may have been due to the higher elevation, or a result of differences in site management. Salal tends to predominate on lower fertility sites and would be less likely to occur in soils subjected to fertilization, which had occurred in the Green River stand during the former stand rotation. Among weedy species typical of clearcuts, fireweed (*Epilobium angustifolium* (L.) L.) was present at all three sites. Only Green River had pearly-everlasting (*Anaphalis margaritacea* (L.) B. & H.) and other composites within the sampling area, although pearly-everlasting and other composites were present, but less common, near the sampling area at the other two sites. An assessment of plants in clearcuts, green-tree retention stands, and forests in the Cedar River watershed (North et al. 1996) found the retention forests to be characterized by a mixture of invasive species (those common to clearcuts) and residual species (plants from the forest understory) similar to the plant community composition at the sites in the present study.

Table 3.2 Understory plant species observed near residual Douglas-fir trees at the three sites.

Green River	Imagine	Beatles
Acer circinatum	*Acer circinatum*	
Alnus rubra	*Alnus rubra*	
Anaphalis margaritacea		
Asarum sp.		
	Blechnum spicant	*Blechnum spicant*
Claytonia sp.		*Claytonia sp.*
Compositae spp.		
Epilobium angustifolium	*Epilobium angustifolium*	*Epilobium angustifolium*
		Equisetum sp.
		Fraxinus brevifolia
Galium triflorum		
	Gaultheria shallon	*Gaultheria shallon*
Holodiscus discolor		
	Linnea borealis	
	Mahonia nervosa	
	Maianthemum dilatatum	*Maianthemum dilatatum*
Polystichum munitum	*Polystichum munitum*	*Polystichum munitum*
Populus trichocarpa		*Populus trichocarpa*
Pteridium aquilinum	*Pteridium aquilinum*	*Pteridium aquilinum*
		Rubus discolor
		Rubus laciniatus
Rubus parviflorus	*Rubus parviflorus*	*Rubus parviflorus*
Rubus spectabilis	*Rubus spectabilis*	*Rubus spectabilis*
Rubus ursinus	*Rubus ursinus*	
		Salix sitchensis
		Smilacina racemosa
		Trillium ovatum
	Vaccinium membranaceum	*Vaccinium membranaceum*
	Vaccinium ovatum	
Vaccinium parvifolium	*Vaccinium parvifolium*	*Vaccinium parvifolium*
Verbascum thapsus		*Verbascum thapsus*
		Viola glabella

3.2.2 Sampling design and field measurements

Douglas-fir seedlings used for the study were three years old at the time of planting. Seedlings grown under standard nursery practices were used which were colonized by mycorrhizal fungi. This more closely mimics normal private and public forestry practices. One Douglas-fir tree which had been retained during harvesting was selected to be the center tree at each of the three sites. Preliminary sampling determined that the roots of each center tree extended no more than 8 m from the base of the tree (data not shown). In April 1998, seedlings were planted within the area defined by concentric circles 2 to 6 m from the base of the center tree and designated as "<6 m" seedlings. ">16 m" seedlings were planted from 16 to 30 m from the center tree. At each center tree, 40 <6 m and 40 >16 m seedlings were planted at a spacing of at least 1 m from each other and from previously established Douglas-fir seedlings at the site. Where necessary, understory vegetation was cleared to provide space for the planted seedlings. Root volumes were determined by water displacement for all seedlings before planting. Seedlings were immersed in water in a beaker equipped with an overflow spigot. The volume (ml) of displaced water was measured in a graduated cylinder and converted to cm^3.

For each growing season, seedling height (cm) was measured to the base of the apical bud and root collar diameter (cm) was measured with calipers. Seedling roots were sampled in October 1998, June 1999, October 1999, and June 2000 with 8 replicates for each of the proximity groups at each of the three sites for the first three samplings. The final sampling was limited to 4 replicates for each proximity group at each site, due to loss of seedlings to mountain beaver damage at Green River. Seedlings were excavated, transported back to the laboratory, and stored at 4° C. After removing mycorrhizal root tips for analysis, the subsampled feeder roots were combined with the remaining root system and dried at 45° C to constant weight to determine the root biomass (g) for each seedling.

In June 2000, for each center tree, the nearest major root system was excavated out from the base of the tree in the direction of four random azimuths to a distance of 2 m, and feeder roots were randomly sampled from that section of the root system and returned to the laboratory for mycorrhizal analysis. Excavation was necessary due to the limited number of mycorrhizal root tips obtained from soil coring, and uncertainty as to the identity of the tree host for roots obtained from soil cores.

3.2.3 Analysis of mycorrhizas

Seedlings were kept refrigerated with roots attached to the shoot for a period not exceeding 4 weeks before mycorrhizal analysis. The root system was gently washed to remove excess soil, then feeder roots were selected at random, excised and immersed in water in a shallow tray over a 1 cm square grid and examined under a 8-40x dissecting microscope. At each of the 100 grid points, the nearest root tip was sorted into preliminary categories of inactive or necrotic, non-mycorrhizal, and mycorrhizal. Root tips lacking a visible mantle were presumed to be mycorrhizal and sampled for molecular analysis unless abundant root hairs were present. Root tips were considered inactive or necrotic if the entire root tip was shrunken and dessicated or if the root cortex was partially or completely decayed or absent, leaving only the stele. Mycorrhizal root tips were sorted into broadly defined morphotypes based on morphological characteristics including branching structure and shape, mantle color and texture, and emanating hyphae and rhizomorphs based on described methods (Ingleby et al. 1990; Agerer 1991; Goodman et al. 2000). Root tips from each morphotype for each seedling or each root sample were counted and lyophilized to prepare for long-term storage and/or DNA extraction.

3.2.4 Molecular analysis

DNA extraction. DNA was extracted from lyophilized mycorrhizal root tips using a modification of the method described by Gardes and Bruns (1993). A single root tip or an approximately 10 mg piece of a larger cluster was placed in a 1.5 ml plastic centrifuge tube, immersed in liquid nitrogen for rapid freezing, and ground to a fine powder using a plastic micropestle (Sigma, St. Louis MO). An equal volume of 2x CTAB lysis buffer

(100 mM Tris-Cl pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2.0 w/v Hexadecyltrimethyl-ammonium bromide) was added to the powder and mixed using the micropestle. The mixture was again immersed in the liquid nitrogen allowed to thaw and mixed again. Three cycles of freeze-thawing were performed by immersing the tube first in liquid nitrogen, then in a 65° C waterbath. The DNA extract was incubated at 65° for 30-60 minutes. Chloroform extraction was performed by adding an equal volume of a 24:1 mixture of chloroform and isoamyl alcohol, vortexing 30 seconds on the lowest setting, and centrifuging at room temperature at 12,500 rpm for 15 minutes. DNA in the supernatant was precipitated by adding an equal volume of cold isopropyl alcohol, incubating on ice for one hour, then centrifuging at 6000 rpm for 5 minutes. The pellet was washed with 50 μl of wash buffer (76% ethanol, 10 mM NH_4OAc) and centrifuged for 15 minutes at 12,000 rpm. After a cold 95% ethanol wash the pellet was air dried at room temperature and resuspended in 30 μl TE (10 mM Tris, 1 mM EDTA, pH 8.0). Extracted DNA samples were stored at –40° C for future analysis.

Restriction fragment length polymorphism (RFLP) analysis. RFLP analysis utilizes restriction endonucleases that recognize short sequences to cleave DNA at specific sites, generating patterns that vary based on the underlying DNA sequence of a gene or gene segment. RFLPs have been used successfully to distinguish among EMF taxa, often to the species level (Egger and Fortin 1990; Gardes and Bruns 1996; Karen et al. 1997; Byrd et al. 2000; Grogan et al. 2000a; Glen et al. 2001). Identifying each EMF taxon relies upon matching RFLP patterns to those obtained from sporocarps of known taxa, a process which requires an extensive RFLP database and often leaves many taxa unidentified (Bruns et al. 1998; Horton and Bruns 2001). In this study, RFLP analysis was used to distinguish among EMF taxa, while identification was accomplished by determining the sequence of multiple genes for each taxon, a process suggested by Horton and Bruns (2001).

In initial sampling, RFLPs from multiple root tips from each morphotype from each seedling were screened and found to be identical. In later sampling, a single root tip was randomly subsampled from each morphotype for each seedling. Because multiple seedlings had the same EMF taxa, this generated a substantial number of RFLP patterns for each taxon. In total, 8905 mycorrhizal root tips were examined for this study, and 700 were selected for DNA extraction and RFLP analysis.

To generate RFLPs, polymerase chain reaction (PCR) was used to amplify the internal transcribed spacers of the nuclear ribosomal RNA gene, using the fungus-specific primer pair ITS-1F (Gardes and Bruns 1993) and ITS-4 (White et al. 1990). The final reaction mixture consisted of a 1:1000 dilution of the DNA extract, 200 μM each of dATP, dTTP, dCTP, dGTP, 200 nM of each primer, 3 mM $MgCl_2$, 0.5 mg/ml of sterile bovine serum albumin, 0.05 units/μl of Taq polymerase (various suppliers), and the standard PCR buffer as supplied by the manufacturer, in a final reaction mixture of 25 μl. Sterile water was used as a negative control, and DNA from *Tricholoma saponaceum* was used as a positive control for each run of PCR. Amplification was performed with a denaturation of 95° C for 35 s, an annealing temperature of 55° C for 55 s, and an increasing extension period of 72° C for 45 s plus 4 s/cycle over 36 cycles. Amplified products were digested with the restriction enzymes Hpa II, Cfo I, and Rsa I, separated on 2% agarose gels, stained with ethidium bromide, and digitally photographed for band analysis using the GelCompar II software package (Applied Maths, Inc., Austin TX, 1991). Gel images were processed to eliminate distortion and bending of the gel, and band sizes were calibrated by comparison with a standard DNA ladder. Cluster analysis was performed by GelCompar II using UPGMA with the fuzzy dice procedure, with a band position tolerance of 1%, and results were examined and adjusted by hand as needed.

DNA sequence analysis. For each EMF taxon as defined by distinct RFLP patterns based on cluster analysis, sequences were determined that were used to identify closely related known taxa based on sequence homology. A multi-stage approach was utilized, in which sequencing of the ITS rRNA using the primer pair ITS-1F and ITS-4 was followed by sequencing of approximately 360 base pairs (bp) of the mitochondrial large subunit rRNA using primer pair ML5/ML6 described by White et al. (1990) and/or approximately 650 bp of the nuclear large subunit rRNA gene using the primer pair

LR0R/LR16 (Moncalvo et al. 2000) as needed to obtain a reliable identification. These genes were selected because (i) they are variable enough to be phylogenetically informative at the genus and species level, (ii) intraspecific variability is generally minimal, although exceptions exist for particular clades (Glen et al. 2001), and (iii) these genes have been utilized for broad phylogenetic studies (Bruns et al. 1998; Hibbitt et al. 2000; Moncalvo et al. 2000) as well as detailed treatments of smaller clades (Feibelman et al. 1997; Aanen et al. 2000; Grubisha et al. 2001; Matheny 2003) and therefore sequence data can be obtained from publicly accessible databases for a large number of fungus taxa.

The National Center for Biotechnology Information web-based BLAST search engine was used to identify closely matched sequences from global databases including GenBank (Altschul et al. 1997). PHYLIP version 3.6a3 (Felsenstein 2002) was used to generate neighbor-joining, parsimony, and maximum-likelihood trees to examine the phylogenetic placement of unidentified fungus taxa with reference to published sequences obtained through BLAST searches as well as sequences obtained from EMF sporocarps collected at the study sites. Sequence homology of 98% or greater for the ITS region and 99% or greater for the nLSU rRNA gene were considered sufficient to assign tentative species-level designations to unidentified fungus taxa.

3.2.5 Calculation of root and shoot biomass and relative growth rates

Initial root biomass was estimated from root volumes measured at planting based on the following relationship:

$$\text{Root biomass (g)} = (0.229) \times \text{root volume (cm}^3)$$

This was derived from the relationship between root biomass measured for a subsample of seedlings sacrificed at planting and their root volumes, using linear regression with the intercept set to zero ($F_{(1,8)}=36.5$, $p=0.001$, $R^2=0.839$). Shoot biomass was directly measured in fall 1999 and spring 2000, but not in fall 1998, and could not be measured at planting due to the destructive nature of the sampling required. Therefore, initial and fall 1998 shoot biomass was estimated as the following:

$$\ln(1 + \text{Shoot biomass}) = (1.020) \times \ln(1 + \text{stem volume})$$

This was derived from the relationship between stem volume and shoot biomass for seedlings measured fall 1999 and spring 2000, using linear regression with the intercept set to zero ($F_{(1, 65)}$=20885, p=0.000, R^2=0.997) with a natural log transformation of both variables to achieve equal variance of the residuals. Root to shoot ratios were calculated as the root biomass (g) divided by the shoot biomass (g). Stem volume and relative growth rates were calculated following van den Driesche (1992), using the allometric equations:

$$\text{Stem volume} = (\pi \times \text{diameter}^2 \times \text{height}) / 12$$

and

$$\text{Relative growth rate} = \frac{\ln w_2 - \ln w_1}{t_2 - t_1}$$

where diameter and height were measured in cm, t was the date measured in years, and w was either root biomass (g) or stem volume (cm^3) for root and stem growth rates, respectively. Heavily browsed seedlings, defined as those which suffered a decrease in height in consecutive measurements, were excluded from the analysis of growth rates. Relative abundance of each EMF taxon identified by RFLP analysis was calculated as the number of root tips with that taxon divided by the number of mycorrhizal tips, on a seedling by seedling basis.

3.2.6 Statistical analyses

For stem growth rates repeated measures ANOVA was performed, while for root growth rates, multi-factor ANOVA was used to test for effects of sample date, site, soil substrate, and proximity to center trees. Stepwise linear regression was used to test relationships between relative abundance of EMF taxa and seedling growth rates. Variables with significant correlations with seedling growth were entered into general linear models (SPSS GLM procedure) as covariates to test their effect with site and proximity as factors. Taxon relative abundance was square-root transformed to address departures from normality, and weighted least squares analysis was used as needed to satisfy assumptions of homogeneity of variance for ANOVA. Post-hoc testing was performed using Tukey's honestly significant difference. Unless otherwise specified, statistical analysis was performed using SPSS for Windows 10.0.5 (SPSS Inc., Chicago, IL, 1999).

3.3 Results

3.3.1 Mycorrhizal status of seedlings

A total of 50 distinguishable EMF taxa were observed on field seedlings at the three study sites (Table 3.3). On four sampling dates starting fall 1998 and ending spring 2000, a total of 5600 root tips from 56 seedlings were examined at each site, of which 2876 root tips had viable mycorrhizas at Green River, 2817 at Imagine, and 2717 at Beatles. No endomycorrhizal fungus taxa were detected through molecular methods on root tips colonized by ecto- (or ectendo-) mycorrhizal fungi in this study, although their presence could have been masked by the more abundant ectomycorrhizal DNA. Root tips with root hairs but no visible mantle could have been colonized by endomycorrhizal fungi, but I did not test for their presence.

The proportion of root tips that were ectomycorrhizal (percent ECM) remained at a steady level for seedlings <6 m and for seedlings >16 m from center trees throughout the study period, ranging by sample date from 51 to 62% of total tips for <6 m seedlings and from 42 to 47% for >16 m seedlings (Table 3.4). These values were low due to the high proportion of root tips designated as necrotic, a category defined primarily based on partial or complete decomposition or loss of the root cortex for a given root tip. Most necrotic tips appeared to have been mycorrhizal, but identification of the EMF taxon was no longer possible. Non-mycorrhizal root tips, distinguished by abundant root hairs, consisted predominantly of newly formed branch roots located near the growing tip of the root. For seedlings near center trees, an average of 34% of root tips were necrotic and 10% were non-mycorrhizal, while seedlings >16 m from center trees had 44% necrotic and 12% non-mycorrhizal root tips. Greenhouse bioassay seedlings had an even higher

Table 3.3 EMF taxa on Douglas-fir seedlings after planting.

	Field Seedlings			**Greenhouse Seedlings**		
	Green River	Imagine	Beatles	Green River	Imagine	Beatles
Total taxa	29	33	39	10	8	9
Active mycorrhizal root tips	2876	2817	2717	190	139	166
Mycorrhizal fungal taxa						
Rhizopogon spp.	19.24	24.17	34.37		8.89	5.05
Rhizopogon I	15.67	18.04	5.17	12.83	9.80	9.09
Tuber I	14.39	1.13	4.31	9.66	9.96	1.30
Rhizopogon vinicolor	12.24	6.35	6.06	5.72		9.09
Pseudotomentella tristis	9.52	10.14	1.17	0.35		
Cenococcum geophilum	5.05	1.58	2.43	3.58	1.98	0.27
Rhizopogon villosulus	3.74					
Tylospora fibrillosa	2.81		0.89			
Tomentella ellisii gp.	2.75	0.68	1.98	19.45	38.57	22.73
Tomentella stuposa	2.52	0.40	1.30			
Thelephora terrestris	2.28	0.11	1.97	17.64	11.43	20.51
Hebeloma album gp.	1.70			10.00	5.04	
Clavulina I	1.24	1.13	0.99			
Tomentella sublilacina	0.97	1.46	3.22			
Tomentellopsis I	0.97					
Russula nigricans	0.92	9.85	5.75			
Tomentella spp.	0.83		0.29			
Tomentella II	0.82		0.88			
Wilcoxina spp.	0.38	0.30	0.43	17.74	14.33	27.42
Sebacina I	0.36	0.57				
Piloderma fallax	0.29		0.21			
Inocybe sindonia gp.	0.22					
Peziza I	0.21					
Amphinema byssoides	0.20		0.75			
Hymenoscyphus ericae	0.11	0.53	0.50	3.03		
Amphinema I	0.09	0.55				
Inocybe sierraensis gp.	0.07					
Inocybe II	0.04	0.21	0.29			
Boletus zelleri		11.36	1.14			
Tylospora asterophora		2.22	0.20			
Pseudotomentella nigra		2.08				
Melanogaster I		1.53	1.03			
Russula chloroides		1.47				
Atheliaceae I		1.36	0.19			
Russula spp.		0.73	1.47			
Atheliaceae II		0.41	1.68			
Russula sphagnophila gp.		0.38				
Laccaria sp.		0.29	1.17			
*Rhizopogon parksii**		0.22	13.93			4.55
Thelephora I		0.13				
Cortinarius cinnamomeus		0.10	0.11			
Amanita I		0.09	0.98			
Russula bicolor		0.03	1.76			
Lactarius I			1.66			
Truncocolumella citrina			0.92			
Russula xerampelina gp.			0.25			
Piloderma byssinum			0.17			
Macowanites iodiolans gp.			0.15			
Inocybe I			0.12			
Inocybe praetervisa			0.10			
Unidentified	0.37	0.38	0.03	0	0	0

Values are means of relative abundance of fungal taxa per seedling (%), from 1998 to 2000. Putative mycorrhizal fungal taxon names are based on DNA sequence homology to collected sporocarps and/or published sequences. Columns sum to 100%.
*could not be distinguished from *Rhizopogon vinicolor* type 2.

Table 3.4 Proportion of root tips with viable mycorrhizas and number of EMF taxa per seedling for seedlings <6 m or >16 m from center trees.

		Percent ECM per seedling			Taxa per seedling		
Sample date	N[a]	<6 m seedlings[b]	>16 m seedlings[c]	P value	<6 m seedlings[b]	>16 m seedlings[c]	*P* value
Fall 1998	48	54.9 ± 3.9	46.6 ± 3.8	0.138	3.96 ± 0.36	3.13 ± 0.23	0.058
Spring 1999	48	55.6 ± 3.5	43.0 ± 2.9	0.008	3.96 ± 0.36	2.92 ± 0.22	0.017
Fall 1999	48	62.2 ± 3.2	41.8 ± 3.0	<0.001	4.42 ± 0.31	3.79 ± 0.29	0.148
Spring 2000	24	50.1 ± 4.2	42.4 ± 3.4	0.168	3.92 ± 0.40	3.50 ± 0.38	0.457
All dates	168	56.5 ± 1.9	43.6 ± 1.7	<0.001	4.08 ± 0.18	3.31 ± 0.14	0.001

Values are means ± SEs with significance tested by single-factor ANOVA. [a]Total number of seedlings examined for each sample date, [b]planted within 6 m of center trees, [c]planted beyond 16 m from center trees.

proportion of necrotic root tips (57%) while the proportion of non-mycorrhizal root tips was also higher than field seedlings (29%) resulting in a remarkably low percent ECM (15%) (Fig. 3.1a). The mean root biomass for greenhouse seedlings was nearly double that of field seedlings, at 55 g compared to 29 g for field seedlings, which may have helped to compensate for the lower proportion of tips that were mycorrhizal.

Of the 50 putative taxa of mycorrhizal fungi which could be distinguished by molecular methods, 29 taxa were found at Green River, 33 at Imagine, and 39 at Beatles (Table 3.3). The low mycorrhizal fungus species richness on field seedlings from Green River was not related to the effective sample size, since it was also the site with the highest number of viable mycorrhizal root tips (Table 3.3). Five EMF taxa were found only at Green River (although one of these taxa was found on greenhouse bioassay seedlings planted in soil from Imagine), while Imagine had four taxa not found elsewhere, and Beatles had the highest number of unique mycorrhizal fungi with seven taxa not found at the other two sites. Green River and Imagine shared two EMF taxa not found at Beatles; Green River and Beatles shared four taxa not found at Imagine, and Imagine and Beatles were the most similar with eleven taxa in common. Only 15 of the 50 EMF taxa were found at all three sites, and can therefore be considered generalists.

Seedlings grown in the greenhouse in soils from the field sites developed mycorrhizas with EMF taxa that generally have been shown to establish readily from spores or other soil propagules on Douglas-fir (Bledsoe and Tennyson 1982; Castellano et al. 1985; Jackson et al. 1995; Parlade et al. 1996) and other seedlings in the Pinaceae (Baar et al. 1999). Most of the mycorrhizal fungi on the greenhouse bioassay seedlings were found on field seedlings at all three of the sites (Table 3.3). One exception occurred with mycorrhizal fungi from the genus *Rhizopogon* (either *R. parksii* or *R. vinicolor* II) which were common on field seedlings at Beatles and at Imagine to a lesser extent, but were not among the *Rhizopogon* species identified at Green River. The other exception occurred with mycorrhizal fungi from the *Hebeloma album* group, which were originally present on nursery seedlings before planting, and appear to have persisted on field seedlings only at Green River after planting. Of the other mycorrhizal fungi on nursery seedlings, *Rhizopogon* I and the *Tuber* species were well represented on both field and greenhouse seedlings, while the ascomycete *Wilcoxina* (probably including both *W. rehmii* and *W. mikolai*) was one of the most abundant mycorrhizal fungi on greenhouse seedlings, but represented less than 1% of those on field seedlings.

Sample date (F=3.795, P=0.034) and site of origin of the potting soil (F=4.407, P=0.021) were significant factors in explaining variation in numbers of EMF taxa per greenhouse seedling (adjusted R^2=0.251), while soil substrate, stem and root volume at planting, and root biomass over time were not significantly related (GLM, α=0.05). Seedlings grown in soil from Green River had an average of 2.6 EMF taxa per seedling, significantly greater than Beatles at 1.5 taxa per seedling as tested by Tukey's honestly significant difference (P=0.029), with Imagine not significantly different from the other two sites at 1.9 taxa per seedling. Since percent ECM did not differ significantly among sites, changes in mycorrhizal abundance did not account for the observed differences in EMF taxa per seedling among sites. The lower species richness for soil from Beatles when assessed as number of EMF taxa per seedling can be attributed primarily to lower evenness of the community as a whole, since the total of nine taxa observed on seedlings in Beatles soil differed only slightly from the ten observed on seedlings in Green River soil (Table 3.3).

3.3.2 Seedling growth

At planting, field seedlings averaged 49 cm in height and 1.2 cm in diameter, resulting in a stem volume of 18.0 cm^3 based on allometric equations. In spring of 2003, after five growing seasons, remaining saplings averaged 167 cm in height, 2.9 cm in diameter, and 451 cm^3 for stem volume growing near center trees, and 223 cm in height, 3.9 cm in diameter, and 984 cm^3 for stem volume growing >16 m from center trees (Table 3.5). The percent height increment was fairly stable throughout the five years of the study for seedlings both <6 m and >16 m from center trees, however the percent diameter increment declined for 2001-2003 for <6 m seedlings, but remained relatively stable for >16 m seedlings (Table 3.5). The mean stem growth rate decreased over the duration of the study for both <6 m and >16 m seedlings (Table 3.5).

Seedling roots grew rapidly in the first season, then more slowly throughout the rest of the study period (Table 3.6). The root biomass decreased over the first winter, from a mean of 29 g to 22 g in spring 1999, however, the difference was not significant (Tukey's HSD, $\alpha=0.05$) (Table 3.6). The ratio of root biomass to shoot biomass increased from 0.65 in spring 1998 at planting as bare-root seedlings to 0.96 for <6 m seedlings and 0.85 for >16 m seedlings in fall 1998. Over the second year of growth, the root to shoot ratio decreased to 0.48 in fall 1999 and 0.43 in spring 2000 for <6 m seedlings and 0.40 in fall 1999 and 0.38 in spring 2000 for >16 m seedlings (Table 3.6).

3.3.3 Effects of proximity to center trees on seedling mycorrhizal attributes

To evaluate the hypothesis that seedlings near trees exhibit higher levels of mycorrhizal colonization, the proportion of root tips with viable mycorrhizas was assessed for seedlings planted <6 m and >16 m from center trees. Seedlings growing near center trees had the highest percent ECM, at 57, with seedlings >16 m from center trees significantly lower at 44 (Table 3.4). Using general linear modeling, proximity to the center tree was the only factor significantly related to percent ECM, with an F value of 28.3 ($P<0.001$) and an adjusted R^2 of 0.400 for the model, while date, site, soil substrate, and initial seedling size were not significant.

Table 3.5 Stem growth of seedlings planted in spring 1998 <6 m or >16 m from trees.

	N[a]	<6 m seedlings[b]	>16 m seedlings[c]	*P* value
Height (cm)				
Spring 1998	218	47.9 ± 0.8	50.2 ± 0.8	0.047
Fall 1998	216	62.7 ± 1.0	64.4 ± 1.1	0.253
Fall 1999	116	79.9 ± 2.1	88.4 ± 2.4	0.011
Spring 2001	51	108 ± 5.0	128 ± 6.8	0.029
Spring 2003	47	167 ± 10.0	223 ± 15.0	0.005
Diameter (cm)				
Spring 1998	218	1.14 ± 0.02	1.15 ± 0.02	0.788
Fall 1998	216	1.32 ± 0.02	1.34 ± 0.02	0.508
Fall 1999	116	1.76 ± 0.05	1.84 ± 0.04	0.218
Spring 2001	51	2.17 ± 0.11	2.42 ± 0.09	0.074
Spring 2003	47	2.90 ± 0.25	3.90 ± 0.18	0.002
Stem volume (cm^3)				
Spring 1998	218	17.4 ± 0.8	18.7 ± 0.9	0.265
Fall 1998	216	30.4 ± 1.3	32.4 ± 1.4	0.318
Fall 1999	116	69.7 ± 4.8	83.2 ± 5.0	0.058
Spring 2001	51	147 ± 18.6	218 ± 26.5	0.050
Spring 2003	47	471 ± 119	984 ± 130	0.007
Height increment (%)				
Fall 1998	216	32 ± 1	29 ± 1	0.047
Fall 1999	116	27 ± 2	37 ± 3	0.008
Spring 2001	51	34 ± 2	39 ± 2	0.177
Spring 2003	47	58 ± 6	76 ± 6	0.046
Diameter increment (%)				
Fall 1998	216	16 ± 1	17 ± 1	0.618
Fall 1999	116	36 ± 3	36 ± 2	0.941
Spring 2001	51	29 ± 4	34 ± 3	0.198
Spring 2003	47	31 ± 6	59 ± 6	0.003
Stem growth rate (yr^{-1})				
Fall 1998	216	1.06 ± 0.04	1.04 ± 0.03	0.653
Fall 1999	116	0.83 ± 0.05	0.91 ± 0.04	0.346
Spring 2001	51	0.49 ± 0.04	0.58 ± 0.03	0.178
Spring 2003	47	0.45 ± 0.05	0.68 ± 0.05	0.012
Stem biomass (g)				
Fall 1999	48	71.9 ± 7.3	77.1 ± 6.9	0.607
Spring 2000	24	97.2 ± 12.8	95.3 ± 14.2	0.922

Values are means ± SEs with significance tested by single-factor ANOVA. Heavily browsed seedlings were excluded from the analysis. [a]Number of seedlings measured for each date, [b]planted within 6 m of center trees, [c]planted beyond 16 m from center trees

Table 3.6 Root biomass, growth rate, and root to shoot ratio for seedlings planted in spring 1998 <6 m or >16 m from trees.

	N[a]	<6 m seedlings[b]	>16 m seedlings[c]	*P* value
Root biomass (g)				
Spring 1998[d]	238	11.2 ± 0.40	11.1 ± 0.41	0.785
Fall 1998	48	28.0 ± 2.1	29.1 ± 2.4	0.719
Spring 1999	48	24.8 ± 2.7	19.4 ± 2.2	0.126
Fall 1999	48	33.7 ± 3.3	30.1 ± 3.1	0.428
Spring 2000	24	41.1 ± 5.3	34.3 ± 4.9	0.354
All post-planting dates	168	30.6 ± 1.6	27.4 ± 1.5	0.123[f]
Root growth rate (yr^{-1})				
Fall 1998	48	1.64 ± 0.13	1.46 ± 0.16	0.395
Spring 1999	47	0.675 ± 0.073	0.544 ± 0.063	0.181
Fall 1999	47	0.638 ± 0.059	0.713 ± 0.068	0.416
Spring 2000	24	0.624 ± 0.036	0.483 ± 0.065	0.070
All dates	166	0.936 ± 0.068	0.850 ± 0.069	0.050[f]
Root to shoot ratio				
Spring 1998[e]	238	0.659 ± 0.021	0.637 ± 0.028	0.527
Fall 1998[e]	47	0.959 ± 0.071	0.846 ± 0.057	0.221
Fall 1999	48	0.483 ± 0.026	0.400 ± 0.020	0.014
Spring 2000	24	0.432 ± 0.020	0.378 ± 0.025	0.107
All post-planting dates	119	0.658 ± 0.043	0.574 ± 0.038	0.018[f]

Values are means ± SEs with significance tested by single-factor ANOVA. [a]Total number of seedlings measured for each sample date, [b]planted <6 m of center trees, [c]planted >16 m from center trees, [d]root biomass estimated from root volume prior to planting, [e]stem biomass estimated from stem volume, [f]*P* value from general linear model with date and site as factors.

The number of EMF taxa per seedling was assessed to evaluate the hypothesis that seedlings planted near residual trees acquire a more diverse array of mycorrhizal fungi than seedlings far from trees. Greenhouse seedlings also were assessed to compare the inoculation potential of soil propagules in the absence of hyphal linkages. As expected, the greenhouse seedlings exhibited significantly lower mean numbers of EMF taxa than field seedlings at 1.97 taxa per seedling, but proximity to the center tree also had a significant effect, with >16 m seedlings maintaining only 3.28 taxa per seedling compared to 4.11 taxa per seedling near the center tree (Fig. 3.1b). In general linear modeling, treatment group (i.e., <6 m, >16 m, and greenhouse seedlings) was the only factor significantly related to the number of EMF taxa per seedling, with an F value of

26.7 ($P<0.001$) and an adjusted R^2 of 0.225, while date, site, soil substrate, and initial seedling size were not significant (GLM, $\alpha=0.05$). When greenhouse seedlings were not included in the analysis, the proximity effect remained significant (F=12.4, $P=0.001$) but with a significant interaction effect with site (F=5.9, $P<0.001$) with an adjusted R^2 of 0.202 for the model. The proximity effect was strongest at Beatles, while seedlings near center trees at Green River and Imagine had only moderate increases in number of EMF taxa per seedling compared to seedlings >16 m from center trees (Fig. 3.1b). These results supported the hypothesis that seedlings near trees have greater mycorrhizal species richness, although the lower species richness observed could also have been due to the smaller chance of detecting rare EMF taxa as a result of lower mycorrhizal abundance of seedlings >16 m from trees (Fig. 3.1a).

For field seedlings, mycorrhizal species richness increased slightly throughout the study period, from an average of 3.5 EMF taxa per seedling in fall 1998 and spring 1999 to 4.2 in fall 1999 and 3.9 in spring 2000 (Table 3.4). In contrast, greenhouse seedlings supported a lower and steadily decreasing number of EMF taxa, with means of 2.5 taxa per seedling in fall 1998, declining to only 1.5 taxa per seedling in fall 1999 (Fig. 3.1b).

3.3.4 Effects of proximity to center trees on seedling growth

To evaluate the hypothesis that seedlings near trees experience higher levels of competition and therefore exhibit growth inhibition, stem and root growth rates were assessed for seedlings planted <6 m and >16 m from center trees. Shoot development was different for seedlings growing <6 m and >16 m from center trees (Table 3.5). Seedlings growing >16 m from center trees were taller, and this proximity effect became more pronounced over time, although it should be noted that differences already existed at planting, possibly due to inadequate randomization during the planting process (Table 3.5). Height increment is a more sensitive measure of factors influencing height growth, since it takes into account the starting height for each seedling. Here proximity effects were less consistent, with >16 m seedlings showing significantly less height increase in 1998, but more height increase than seedlings near center trees in subsequent samplings.

It was only after completion of mycorrhizal sampling in spring of 2000 that remaining seedlings displayed significant differences in diameter and diameter increment, but by spring 2003 these differences were substantial, with diameters of 3.9 cm for >16 m seedlings compared to 2.9 cm for <6 m seedlings and diameter increases of 59% compared to 31% (Table 3.5). Stem volumes reflected the disparity between <6 m and >16 m seedlings' heights and diameters. By spring 2003, >16 m seedlings had more than double the stem volume of <6 m seedlings at 984 cm^3 compared to 470 cm^3. Over the period from 2001 to 2003, >16 m seedlings had substantially higher mean stem growth rates of 0.68 compared to 0.45 for <6 m seedlings (Table 3.5).

For seedling stem growth rates from spring 1998 to spring 2003, proximity to the center tree was not a significant factor in multi-factor ANOVA (P=0.87), however site effects (F=34.8, P<0.001) and the interaction between site and proximity (F=9.6, P<0.001) were significant, with an adjusted R^2 of 0.644 for the model. The significant interaction term implied that the effect of proximity depended on the site. This result can be explained by growth inhibition experienced by seedlings near the center tree at Beatles, and to a lesser extent at Imagine. Seedlings at Green River experienced the highest stem growth rates throughout the course of the study (Fig. 3.2). Seedlings < 6 m from the center tree experienced significantly higher stem growth rates than seedlings >16 m from the center tree, when sampled in 1999, 2001, and 2003 (Fig. 3.2 b-d). Unfortunately, heavy browsing by mountain beavers denning within the <6 m seedling plot at Green River reduced the sample size to only two surviving seedlings in 2001 and 2003. Nevertheless, the trend was clearly established in fall of 1999 before grazing damage occurred (Fig. 3.2b). Seedlings at Beatles and Imagine grew more slowly than at Green River, but only at Beatles was seedling growth from 1999 on significantly inhibited by proximity to the center tree (Fig. 3.2 b-d).

In contrast to its effect on stem growth rates, proximity to center trees had a stimulatory effect on seedling root development (Table 3.6). In a weighted least squares general linear model, root growth rates were related to sample date (F=29.4, P<0.001), site (F=5.9, P=0.003), and proximity to center trees (F=3.916, P=0.050), with an adjusted

R^2 of 0.397 for the model. Similarly to stem growth rates, root growth rates were significantly higher at Green River (1.05/yr) than at Beatles (0.80/yr, P=0.002) and at Imagine (0.90/yr, P=0.011) while Beatles and Imagine did not significantly differ (P=0.87), by Tukey's honestly significant difference test. Seedling root biomass was lower for seedlings growing >16 m from center trees (Table 3.6), which was notable taking into account the larger stem volume and shoot biomass of >16 m seedlings (Table 3.5). As a result, root to shoot ratios averaged 0.66 for <6 m seedlings but only 0.57 for >16 m seedlings. Proximity was a significant factor in explaining variation in root to shoot ratios (F=5.79, P=0.018) along with sample date (F= 53.69, P=0.000), with an adjusted R^2 of 0.511.

3.3.5 Relationship between mycorrhizal status and seedling growth

Variations in seedling stem growth rates from 1998 to 2000 were significantly related to percent ECM (F=6.69, P=0.012), in addition to site effects (F=3.43, P=0.039) and an interaction between date and percent ECM (F=7.42, P=0.008), using repeated measures general linear modeling. Proximity to the center tree was not a significant factor in explaining seedling stem growth rates (P=0.159) when the variability explained by percent ECM was accounted for. Variations in root growth rates also were significantly related to percent ECM (F=12.68, P=0.000) with date (F=31.44, P=0.000) and site (F=4.34, P=0.015) as significant factors.

Mycorrhizal abundance was associated with faster seedling growth in the first growing season after planting. For stem growth rates in 1998, percent ECM was positively correlated with growth (β=0.310, F=7.054, P=0.009) although the relationship was weak (adjusted R^2 of 0.037), while site (P=0.541) and proximity to center trees (P=0.739) were not significant factors. Percent ECM also was positively correlated with the root growth rate for fall of 1998 (β=0.375, F=7.381, P=0.009, adjusted R^2=0.122) and spring of 1999 (β=0.375, F=7.381, P=0.009, adjusted R^2=0.122).

In contrast, seedling growth rates for the 1999 growing season showed a markedly different pattern: percent ECM was negatively correlated with aboveground growth rates and not significantly related to root growth rates in fall of 1999 and spring of 2000. For stem growth rates in fall of 1999, higher percent ECM (F=12.8, *P*=0.001) was associated with lower growth rates, with significant site effects (F=5.9, *P*=0.005) and an adjusted R^2 of 0.228 for the model, while proximity was not a significant factor (*P*=0.201). Percent ECM was not significantly correlated with root growth rates in fall of 1999 (*P*=0.248) and spring of 2000 (*P*=0.413) (data not shown).

Numbers of EMF taxa per seedling, taxa producing large fruiting bodies (epigeous or hypogeous), taxa found on center trees, and taxa forming rhizomorphs were not significantly related to seedling growth rates in general linear models when site and proximity effects were taken into account (data not shown). Seedling stem and root growth rates also were not significantly related to abundance of the 15 most common EMF taxa (those with greater than 1% relative abundance) when site and proximity effects were taken into account (data not shown).

3.4 Discussion

3.4.1 Mycorrhizal status of seedlings

The total of 50 EMF taxa observed on Douglas-fir field seedlings in this study is higher than the 33 morphotypes observed by Roth and Berch (1992) on Douglas-fir seedlings one year after planting in clearcuts on Vancouver Island in Canada, but falls within the range of total numbers of morphotypes reported for other conifer seedlings (Kranabetter and Wylie 1998; Kranabetter et al. 1999; Kranabetter and Friesen 2002). Seedling mycorrhizal colonization levels in this study were low compared to values reported for other conifer seedlings, which often approach 100% (e.g., Jonsson et al. 1999a; Jones et al. 2002). Kropp (1982) also observed low colonization rates for container-grown western hemlock seedlings. The unusually high proportion of necrotic root tips for greenhouse seedlings may have been a result of the highly constrained rooting space in pots. Most seedlings appeared to become moderately to severely root-bound within the first year of growth. The higher proportion of necrotic root tips for seedlings planted >16 m from trees may reflect a faster fine root turnover rate and warrants further study.

While colonization levels and numbers of EMF taxa per seedling remained steady for field seedlings, greenhouse seedlings experienced a decrease in percent ECM and numbers of EMF taxa per seedling over time. The decrease in EMF taxa could have been due to the lower chance of detecting rare taxa as a result of the decreasing mycorrhizal abundance, but may have been compounded by competitive exclusion of some taxa under the simplified conditions of container cultivation.

The sites Imagine and Beatles had the highest number of EMF taxa in common. This was probably due to similarities in time since harvesting and the age of residual trees. The forest at Green River was also higher in elevation and had been fertilized at least once during the last stand rotation, which would tend to favor a distinctive EMF community.

3.4.2 Seedling growth

The mean stem growth rate of seedlings decreased over the five years of the study. This decrease probably reflected growth inhibition due to competition as the stand entered the stem exclusion phase (Oliver and Larson 1996). The mean root biomass decreased from fall 1998 to spring 1999. This could have resulted from winter die-back of fine roots combined with the lingering effects of transplantation shock. The ratio of root biomass to shoot biomass nearly doubled over the first growing season, then decreased in the second year of growth. The initial increase in the ratio probably occurred as roots proliferated to establish a root system sufficient to provide necessary soil resources. The decrease in the root to shoot ratio in the second year probably reflected greater allocation of resources to aboveground growth in the competition for light as seedling canopies began to overlap each other in the regenerating stand. A study of Douglas-fir seedling growth in southwestern Oregon reported similar patterns of increasing root to shoot ratio one year after planting, decreasing to around 0.5 by the third year after planting (Tesch et al. 1993).

3.4.3 Effects of proximity to center trees on seedling mycorrhizal attributes

Seedlings < 6 m from residual trees acquired or retained a higher percent of EMF root tips than those >16 m from trees. Dickie et al. (2002) also found this with red oak seedlings planted near stump-sprouting chestnut oak (*Quercus montana*) trees as did Onguene and Kuyper (2002) with ectomycorrhizal *Paraberlinia* seedlings in Cameroon. Hagerman et al. (1999b) found that Engelmann spruce seedlings planted in the root zone near forest/clearcut edges had higher rates of mycorrhizal colonization than seedlings planted in clearcuts, however, the edge effect was prevented by mechanical site-mounding (Jones et al. 2002). Zhou et al. (1998) also found higher mycorrhizal colonization rates in northern red oak seedlings planted in stands with partial canopy removal in comparison to clearcuts.

In this study, seedlings < 6 m from center trees had more EMF taxa than seedlings >16 m from center trees. These results are consistent with several other studies of the effects of proximity to trees on seedling mycorrhizal diversity (Deacon and Donaldson 1983; Kranabetter and Wylie 1998; Kranabetter 1999; Hagerman et al. 1999b). The observed EMF taxon richness for <6 m seedlings (4.11) was similar to the 4.3 types per seedling reported by Simard (1995) for Douglas-fir seedlings growing under Douglas-fir trees, while seedlings prevented from hyphal linkages to outside trees by trenching had only 2.5 types per seedling, more similar to the 1.97 EMF taxa per seedling observed for greenhouse seedlings in the present study.

3.4.4 Effects of proximity to center trees on seedling growth

Effects of center trees on seedling growth were complex: seedlings < 6 m from center trees had increased root growth over the period from 1998 to 2000, while stem growth rates were increasingly inhibited over the study period. Effects of proximity to trees on seedling stem growth were dependant on site conditions. Stem growth rates were higher for seedlings < 6 m from the center tree at Green River, where understory development was minimal. This effect was established by fall 1999, but can only tentatively be supported for 2001 to 2003 due to the unfortunate loss of most of the <6 m seedlings at the site to localized heavy browsing by mountain beavers.

Green River was very open compared to the other two sites, due partly to the more recent harvest, but also because the stand before harvesting was apparently quite dense, with very little understory development, judging from adjacent unharvested stands. Non-study seedlings planted by Plum Creek Timber Company in the year after harvesting appeared to have experienced relatively high mortality, resulting in a low stocking density of non-study Douglas-fir seedlings before the study seedlings were planted. In contrast, both Beatles and Imagine had a well-developed understory at the time of harvest, again judging by adjacent unharvested stands. The understory consisted of western hemlock seedlings or saplings as well as understory shrubs including salmonberry, thimbleberry and blackberry species, and, especially at Imagine, salal. Imagine, in particular, had dense thickets of advance regeneration western hemlock seedlings in the area where >16 m seedlings were planted, so that seedlings in both treatment groups may have experienced similar levels of competition.

The more extensive period after harvesting before the commencement of this study allowed understory plants to proliferate and gave a critical head-start to non-study Douglas-fir seedlings planted after harvesting, especially at Beatles, where they had commonly attained heights of 5 m by 2003. Although study seedlings were placed a minimum of several meters from the nearest non-study Douglas-fir seedlings or saplings to avoid interaction with the root system, the planting scheme was designed to be effective only for the duration of the mycorrhizal study through spring 2000, therefore seedling growth from 2000 to 2003 would have been subjected to progressive degrees of shading and root zone competition.

In this study, seedlings >16 m from center trees allocated more resources to aboveground growth, while seedlings < 6 m from center trees had greater root development at the expense of shoot growth. Zhou et al. (1998) found that red oak seedlings planted in partial canopy retention sites also had higher root to shoot ratios than seedlings in clearcuts. This effect may be related to the presence of residual trees as opposed to competition *per se*, since these results conflict with a series of studies in southwestern Oregon in which increased levels of competition between Douglas-fir seedlings and shrubs (Tesch et al. 1993), grasses and other herbaceous understory plants, other tree species, and neighboring Douglas-fir seedlings (Newton and Cole 1991; Shainsky et al. 1992) resulted in decreases in both root and shoot biomass but did not change the root to shoot ratio.

Changes in the ratio of root and shoot biomass could be related to competition for soil resources. Vogt et al. (1987) found that fine root biomass of Douglas-fir stands was higher at low productivity sites. Seedlings near trees may experience reduced availability of water and soil nutrients due to competition with mature tree roots. Sparks (2004) found that soil trenches which excluded mature tree roots from soil plots near forest edges resulted in significant increases in soil moisture and more rapid growth of western hemlock (but not of Douglas-fir) seedlings. Trenching resulted in increased survival rates of ectomycorrhizal (but not of arbuscular mycorrhizal) seedlings growing in a mixed temperate forest (Booth 2004). Simard (1997d) found increased soil moisture due to soil trenching in September but not in August, however, with no effect on soil nutrients including various forms of nitrogen, and no differences in Douglas-fir seedling growth due to trenching.

3.4.5 Relationship between mycorrhizal status and seedling growth

Over the first two growing seasons in this study, the effect of tree proximity on seedling stem growth rates was no longer significant once the variability explained by percent ECM was accounted for. These results suggest that proximity to trees may affect seedling growth rates through factors influencing mycorrhizal abundance, at least in the first few years after planting. There is some evidence that hyphal linkages to trees and other plants can enhance mycorrhizal colonization rates (Massicotte et al. 1994; Simard et al. 1997d; Kranabetter 1999; Hagerman et al. 1999b; Dickie et al. 2002; Onguene and Kuyper 2002) but mycorrhizal abundance also could be impacted by the turnover rate of mycorrhizal root tips. The high proportion of necrotic root tips observed in the course of this study, and the greater proportion of necrotic root tips on seedlings >16 m from center trees, would support this interpretation. Hyphal linkages to mature trees have been proposed to provide an essential extra source of energy for colonization of seedlings by mycorrhizal fungi taxa with high energy demands, referred to as "late-stage" fungi (e.g., Deacon and Donaldson 1983; Fox 1986). Seedlings near center trees may have been able to maintain a higher percent ECM due to energy subsidies provided by hyphal linkages.

Seedling stem growth rates were related to percent ECM positively in the first year but negatively in the second year of growth. Maintenance of more mycorrhizas might have been beneficial to seedlings initially, during the critical establishment period of the first growing season, but might have reduced aboveground growth of seedlings over the second season, perhaps by costing the seedlings more in photosynthate than the benefit provided by increased access to soil resources. Results are mixed from other studies of the effects of mycorrhizal abundance on growth. Roth and Berch (1992) reported no correlation between Douglas-fir seedling growth and levels of mycorrhizal colonization one growing season after outplanting. Similarly, Flynn et al. (1998) found no correlation between naturally regenerated Sitka spruce seedling growth and mycorrhizal colonization levels. However, other studies have shown a positive relationship between colonization levels and growth for container-grown Douglas-fir (Borchers and Perry 1990) and for other ectomycorrhizal tree seedlings in field studies (Christy et al. 1982; Dickie et al. 2002; Onguene and Kuyper 2002). In contrast, Wu et al. (1993) observed a negative correlation between levels of mycorrhizal colonization and growth over the five years after outplanting for red pine seedlings. Zhou et al. (1998) observed a positive correlation between percent ECM and biomass for red oak seedlings in the first season after planting, but a negative correlation in the second growing season, similar to the results of the present study. The variability in response of seedlings to levels of EMF colonization from study to study is not surprising considering that differences in soil fertility, moisture availability, and temperature would be expected to strongly influence the relative benefit for seedlings. Seedling growth response might also depend on functional differences among EMF taxa.

Mycorrhizal colonization levels also have shown inconsistent effects on root to shoot ratios in other studies. An association between higher mycorrhizal colonization levels and shifts in resource allocation to roots rather than shoots has been observed for axenically grown inoculated gray birch seedlings (Baxter and Dighton 2001), for field grown red oak seedlings, especially when shaded (Zhou et al. 1998), and for subalpine fir and lodgepole pine seedlings naturally regenerating after wildfire (Miller et al. 1998). In contrast, field-grown valley oak (*Quercus lobata*) seedlings had increased shoot growth and decreased root growth after soil transfers which increased mycorrhizal infection and mycorrhizal diversity (Berman and Bledsoe 1998). Part of the difficulty in interpreting these patterns may be the influence of other factors such as various levels of above and below-ground competition in the different studies.

The lack of a relationship between stem and root growth rates and numbers of EMF taxa per seedling was interesting in light of other studies which have experimentally manipulated EMF diversity, linking increased diversity to increased plant productivity. Productivity of calcareous grassland plant species increased significantly with higher arbuscular mycorrhizal fungus diversity in artificial macrocosms with calcareous grassland plant species (van der Heijden et al. 1998). For scotch pine and European white birch seedlings grown in sterile culture and inoculated with up to eight EMF taxa, higher mycorrhizal diversity increased seedling productivity substantially, but only on low fertility substrates (Jonsson et al. 2001). Mycorrhizal diversity has been proposed to aid plants in responding to changing environmental conditions (Jones et al. 2003), for example due to drought (Bledsoe 1986) or anthropogenic disturbance (van der Heijden et al. 1998). The relationship between mycorrhizal diversity and seedling growth has rarely been examined in field settings, however. One of the few field studies found no relationship between numbers of mycorrhizal morphotypes per seedling and growth of Sitka spruce seedlings in Scotland (Flynn et al. 1998).

It was noteworthy that the present study did not support the hypothesis that seedling growth is influenced by the relative abundance of functional groups of EMF taxa defined based on type of emanating hyphae, using the categories described by Agerer (2001). In particular, EMF taxa forming rhizomorphs have been proposed to be more effective at long distance transport of water (Parke et al. 1983) and are thought more likely to form hyphal linkages between individual trees (Read et al. 1985; Simard 1995), but the proportion of rhizomorph-forming taxa per seedling had no significant relationship to growth in this study.

Similarly, the lack of a relationship between growth and the proportion of EMF taxa shared with center trees lends no support to the possibility that seedlings were acquiring resources from adjacent trees via hyphal linkages in quantities sufficient to affect growth. Nevertheless, carbon subsidies through mycorrhizal hyphal linkages could be masked by effects of competition with mature trees. In a large-scale field study, Booth (2004) distinguished between effects of common mycorrhizal networks and root competition on seedling survival and growth by excluding roots but not hyphae in comparison to trenching. Eastern white pine seedlings had increased needle production when root competition was prevented, but when hyphal linkages were allowed in the absence of root competition needle production was even greater (Booth 2004). In mixed Douglas-fir/birch forests in British Columbia, Simard et al. (1997c) demonstrated net carbon transfer from paper birch seedlings to Douglas-fir seedlings of 3% to 10% of the total carbon fixed, with the greatest net transfer to shaded seedlings.

It was also surprising that, while *Rhizopogon* species constituted over half (54 ± 2.4%) of the mycorrhizal root tips observed on field seedlings in this study, with the relative abundance ranging from 0% to 100% for the 159 seedlings sampled, there was no apparent relationship between abundance of *Rhizopogon* species and growth of individual seedlings. In Oregon, Castellano and Trappe (1985) observed growth stimulation for Douglas-fir seedlings outplanted after inoculation with *Rhizopogon* species when compared to seedlings colonized by indigenous fungi. In contrast, Berch and Roth (1993) observed increased growth of nursery seedlings after inoculation with *Rhizopogon vinicolor*, but no growth differences after outplanting on Vancouver Island, Canada, which the authors proposed to be due to browse damage. It is possible that cases of observed growth stimulation are due to increased levels of mycorrhizal colonization before outplanting, as opposed to any intrinsic benefit from particular EMF taxa over indigenous taxa acquired after outplanting.

3.5 Conclusions

Seedlings planted after harvesting are affected in a variety of ways by proximity to residual trees. Root growth rates were enhanced by proximity to trees, especially in the first year after planting. Stem growth was inhibited for seedlings < 6 m from trees, with differences in height, diameter, and stem volume apparent by the third year after planting. Seedling growth inhibition over the five years after planting depended on site, however, with the effect possibly related to density of competing understory vegetation. Seedlings < 6 m from residual trees appeared to divert more resources towards root development over shoot development, developing higher root to shoot biomass ratios than seedlings >16 m from trees. Seedlings < 6 m from residual trees sustained EMF communities that were both more abundant and more diverse, which could impact their response to future fluctuations of environmental conditions. Seedlings < 6 m from trees sustained a greater proportion of active mycorrhizal roots, which was correlated with increased root and stem growth in the first year after planting, but with decreased stem growth in the second year. While the long-term costs and benefits of proximity to residual trees for Douglas-fir regeneration can be established only through extending the study period beyond the scope of this project, these results support residual forestry as a viable alternative to clearcutting.

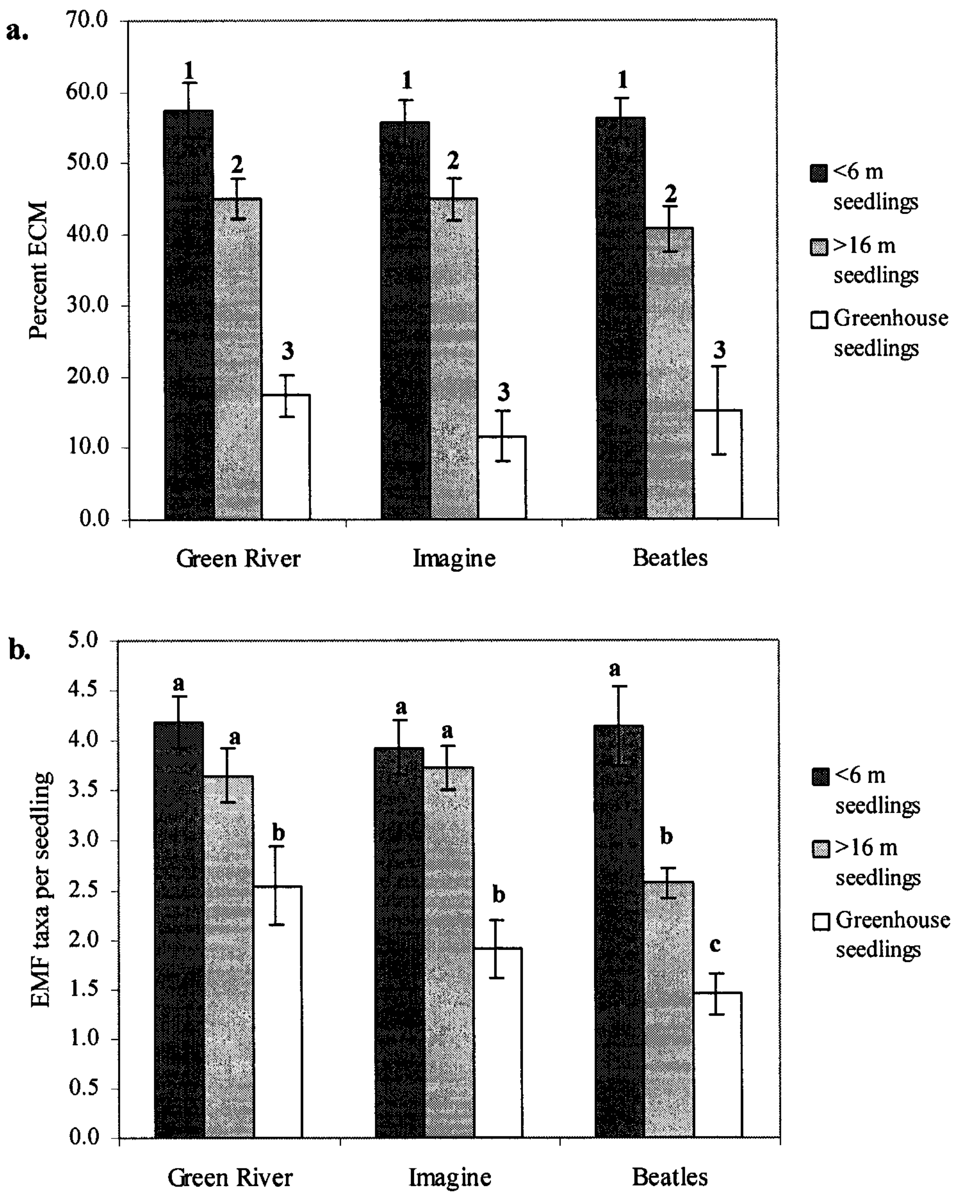

Fig. 3.1 Seedling EMF a) abundance and b) species richness, compared by site and by seedling treatment group.

Values are means ± SEs. Seedlings were planted < 6 m or >16 m from center trees. Greenhouse seedlings were potted in field soils. *P* values are for single-factor ANOVA analyses amongst treatments. For each site, means with the same letter are not significantly different amongst treatments by Tukey's honestly significant difference test. Number of seedlings sampled: 84 <6 m seedlings, 84 >16 m seedlings, and 34 greenhouse seedlings.

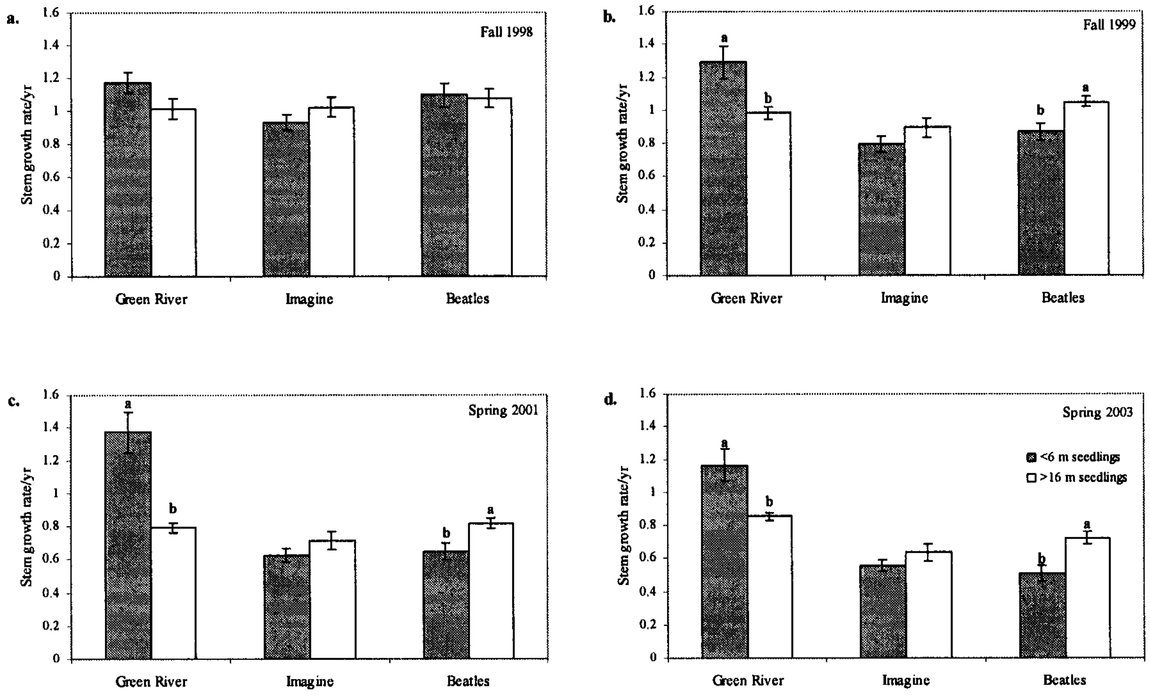

Fig. 3.2 Field seedling stem growth rates.

Values are means ± SEs of natural log of change in biomass per year from planting in spring 1998 until a) fall 1998, b) fall 1999, c) spring 2001, and d) spring 2003. Seedlings were planted < 6 m or >16 m from center trees. *P* values are for single-factor ANOVA comparisons of <6 m and >16 m seedlings at each site. Number of seedlings sampled: 216 in fall 1998, 123 in fall 1999, 51 in spring 2001, 47 in spring 2003.

Chapter 4. Mycorrhizal Fungus Communities of Douglas-fir Seedlings: Effects of Proximity to Mature Trees

4.1 Introduction

Concern over the low structural diversity and reduced species richness of managed Douglas-fir forests in the Pacific Northwest has led to the proposal that clearcutting be replaced by retention forestry (Kohm and Franklin 1997). Retention forestry, also known as green-tree retention, requires some proportion of the mature trees to be protected during harvesting. Residual trees may be evenly spaced (dispersed retention) or clumped to create tree 'islands' or protect sensitive areas by creating buffer zones, for example along stream banks (aggregated retention). Residual trees have been demonstrated to reduce erosion, moderate the effects of wind and solar exposure, and increase diversity in forests recovering from harvesting by retaining some forest-dependant plant and animal species (Perry et al. 1989a; North et al. 1996). Retention of green trees, snags, and logs is now mandated by private (WFPA 1995) and public (FEMAT 1993; WFPB 1995) forest management plans in the Pacific Northwest. The effectiveness of retention forestry in maintaining EMF diversity, however, has not been fully evaluated.

Forest harvesting affects EMF by changing the age structure and species composition of the forest overstory trees and understory plants which serve as their hosts and by modifying the physical, chemical, and biological components of their soil habitat. Several researchers have reported declines in diversity for EMF after forest harvesting. Byrd et al. (2000) found lower species richness and significant changes in EMF community composition for 8-year-old lodgepole pine clear-cuts in Wyoming, when compared to unharvested sites. In a mixed subalpine fir and Engelmann spruce forest in Canada, Hagerman et al. (1999a) also observed declines in diversity of EMF in soil cores after clearcutting, but declines were less severe within 2 m of the forest edge. Likewise, Stockdale (2000) observed a 23% decline in EMF taxa, distinguished morphologically, after thinning of a late-successional Douglas-fir forest in Oregon, while soil cores from within the dripline of residual trees showed no decline in species richness. Impacts of forest clearcutting and thinning on mycorrhizal communities could result from loss of inoculation potential, decreases in inputs of carbon from host plants, changes to the soil environment, or a combination of these factors (Jones et al. 2003).

Several recent studies have documented edge effects on EMF diversity of seedlings planted in clearcuts adjacent to unharvested forest. Engelmann spruce (non-mycorrhizal) bioassay seedlings planted within 2 m of forest edges had higher EMF species richness than seedlings planted 16 m into clearcuts (Hagerman et al. 1999b). In contrast, proximity to the forest edge had no effect on previously colonized Engelmann spruce seedlings planted in mineral soil exposed by mechanical mounding at the same site (Jones et al. 2002). Previously colonized western hemlock and lodgepole pine seedlings had decreased EMF species richness per seedling when planted beyond 7 m from the forest edge into clearcuts (Durall et al. 1998). The same was true for naturally regenerated western hemlock seedlings near the edge of mature forests (Kranabetter and Wylie 1998). When seedlings from the forest were transplanted into clearcuts, EMF diversity declined due to the loss of several taxa which were apparently unable to persist in forest openings (Kranabetter and Friesen 2002). It is not clear from these studies whether changes in the soil environment or loss of hyphal linkages are responsible for the lower EMF species richness of seedlings in clearcuts, but availability of inoculum does not appear to be the primary factor (Jones et al. 2003).

The few studies that have examined the mycorrhizal diversity of seedlings growing near isolated trees have found effects similar to those conferred by forest edges. Proximity to mature paper birch trees resulted in higher mycorrhizal diversity of paper birch seedlings both in clearcuts and in mixed conifer forests (Kranabetter 1999). Dickie et al. (2002) demonstrated that red oak seedlings growing near harvested, stump-sprouting (living) oak trees had greater mycorrhizal colonization and diversity than seedlings near dead oak stumps. Hyphal linkages to mature trees may be critical for maintaining some EMF taxa on seedlings, possibly due to high demand for photosynthate (Fleming 1983; Fleming 1984). Soil trenches dug around Douglas-fir seedlings in 90- to

120-year-old Douglas-fir/paper birch forests resulted in decreases in EMF species richness, and taxa forming rhizomorphs decreased sharply in abundance (Simard et al. 1997d).

The objective of this study was to assess the spatial influence of residual trees on EMF communities of Douglas-fir seedlings. The following hypotheses were tested: (i) the EMF community of seedlings growing near isolated mature trees will be more diverse than that of seedlings far from trees, (ii) EMF taxa associated with mature trees will be more common on seedlings near mature trees, and (iii) EMF taxa able to colonize seedlings from soil propagules (i.e., those present on greenhouse seedlings) will be more common on seedlings far from mature trees.

4.2 Materials and methods

4.2.1 Site characteristics

This study took place in recently harvested second growth Douglas-fir forests in the foothills of the western Cascade mountain range in Washington state, USA, approximately 50 km southeast of the city of Seattle. Two sites, "Beatles" (47° 20.970' N, 121° 49.895' W) and "Imagine" (47° 23.956' N, 121° 48.938' W) were on public land managed by the City of Seattle Cedar River watershed while one site, "Green River" (47° 18.719'N, 121° 42.609' W) was owned and managed by Plum Creek Timber Company. The sites were between 7 and 13 km from each other, and have been described in detail in Chapter 3. Sites were selected based on the presence of mature Douglas-fir trees sufficiently isolated to allow seedlings to be planted in circular bands within 6 m from the center tree and beyond 16 m from the center tree, without approaching within 16 m of neighboring trees. Site characteristics and attributes of each center tree were presented in Chapter 3 in Table 3.1.

Before harvesting, forests at the sites were dominated by Douglas-fir, but with young and mature western hemlock also common at Beatles and Imagine. Other tree species present at or near the sites were western red cedar, Pacific silver fir, grand fir, Sitka spruce (*Picea sitchensis* (Bong.) Carr.), vine maple, red alder, black cottonwood (*Populus trichocarpa* (L.) T. & G.), and Sitka willow (*Salix sitchensis* (L.) Sanson). Green River (808 m elevation) was harvested in 1996, Imagine (457 m) was harvested in 1994, and Beatles (488 m) was harvested in 1992.

4.2.2 Experimental design

In spring of 1998, mycorrhizal nursery-grown bare root 2-1 Douglas-fir seedlings (provided by Plum Creek Timber Co. from their nursery) were planted within circular plots centered upon a single mature Douglas-fir tree at each of the three sites. Around each tree, 40 seedlings were planted in a circular plot within 6 m of the base of the tree and 40 seedlings were planted 16 to 30 m from the base of the tree. Each seedling was planted at least 1 m from the nearest seedling, and previously established understory vegetation was cleared to provide space as needed.

Also in spring of 1998, 16 seedlings were potted in soil from each site. Soil grab samples from 0 to 40 cm depth were merged from four randomly selected locations at the edge of each of the plots to provide a composite sample from each site. Seedlings were grown in a sheltered outdoor area at the University of Washington greenhouse maintained by the Biology department. Water was provided through wicking from below (to avoid leaching of nutrients) by immersing pots in 2 cm of water, in separate saucers to minimize transfer of EMF spores from seedling to seedling. No fertilizers were provided; seedlings were limited to the nutrients present in the soils at sampling.

4.2.3 Sampling of mycorrhizas

Douglas-fir seedlings. In spring of 1998, a subsample of nursery seedlings was taken to determine the initial mycorrhizal composition of seedlings (n=8). Seedlings planted <6 m and >16 m from center trees were sampled in fall of 1998, spring of 1999, fall of 1999, and spring of 2000, with replicate seedlings selected randomly from each plot at each site (2 plots X 3 sites X 8 replicates = 48 seedlings per sample date). In spring of 2000, unusually high seedling mortality due to mountain beavers denning near the plot area at Green River forced reduction of the number of replicates to four. Greenhouse seedlings were sampled in fall of 1998, spring of 1999, and fall of 1999, with four replicates for soil from each of the three sites. Nursery seedlings were small enough that all mycorrhizal root tips could be examined within a reasonable period. For field and greenhouse seedlings, roots were subsampled and 100 root tips were examined from each seedling. Seedlings were carefully excavated to obtain the entire root system. In the laboratory, seedling roots were soaked in distilled water then gently teased apart to break up soil aggregates. Roots were rinsed then placed in shallow trays over a 1 cm grid in random orientations. The root tip closest to each of the 100 evenly-spaced grid points was removed for further analysis.

Mature tree roots. EMF communities of mature Douglas-fir trees were assessed by partially excavating the root system of each center tree. Due to the high degree of soil disturbance involved, excavation could not be performed until the final seedling sample date in spring of 2000, to avoid severing potential hyphal connections between seedlings and the center tree. Four random bearings were taken at each center tree. Along each bearing, a major root (>20 cm diameter) was excavated outwards from the base of each center tree to a distance of 2 m. At this distance, a grab sample of smaller branch roots was taken and returned to the laboratory for analysis. Where necessary to obtain sufficient quantities of fine roots, the excavation was extended up to 4 m from the base of the tree. Mature roots were prepared as described above for field seedlings.

4.2.4 Morphological and molecular identification of EMF taxa

Morphological analysis. Root tips were viewed with a dissecting microscope and sorted into categories of inactive or necrotic, non-mycorrhizal, and mycorrhizal. Root tips lacking a visible mantle were presumed to be mycorrhizal and sampled for molecular analysis unless abundant root hairs were present. Root tips were considered inactive or necrotic if the entire root tip was shrunken and dessicated or if the root cortex was partially or completely decayed or absent, leaving only the stele. Mycorrhizal root tips were sorted into broadly defined morphotypes based on morphological characteristics including branching structure and shape, mantle color and texture, and emanating hyphae and rhizomorphs based on described methods (Ingleby et al. 1990; Agerer 1991; Goodman et al. 2000). Root tips from each morphotype for each seedling or each root sample were counted and lyophilized to prepare for long-term storage and/or DNA extraction.

DNA extraction. DNA was extracted from mycorrhizal root tips using the method described by Gardes and Bruns (1993) with the following modifications: the volume of CTAB lysis buffer was reduced from 300 µl to 40 µl, liquid nitrogen was used instead of a dry ice bath, autoclaved plastic micropestles were used (Sigma, St. Louis MO), and the DNA extract was resuspended in TE (10 mM Tris, 1 mM EDTA, pH 8.0). Extracted DNA samples were stored at –40° C for future analysis.

Restriction fragment length polymorphism (RFLP) analysis. In fall of 1998, for each seedling or mature root sample, RFLPs from multiple root tips of a single morphotype were screened and found to be identical. In later sampling, a single root tip was randomly selected for molecular analysis for every morphotype from each seedling or root sample. In total, over 24,000 root tips were examined for this study, of which 11,856 were active mycorrhizal root tips. A total of 767 root tips were selected for DNA extraction and RFLP analysis. To generate RFLPs, polymerase chain reaction (PCR) was used to amplify the internal transcribed spacers of the nuclear ribosomal RNA gene, using the fungus-specific primer pair ITS-1F (Gardes and Bruns 1993) and ITS-4 (White et al. 1990). The final reaction mixture consisted of a 1:1000 dilution of the DNA extract, 200 µM each of dATP, dTTP, dCTP, dGTP, 200 nM of each primer, 3 mM $MgCl_2$, 0.5 mg/ml of sterile bovine serum albumin, 0.05 units/µl of Taq polymerase (various suppliers), and the standard PCR buffer as supplied by the manufacturer. The volume of the final reaction mixture was 25 µl, overlaid with a single drop of mineral oil (Sigma). Sterile water was used as a negative control, and DNA from *Tricholoma saponaceum* was used as a positive control for each run of PCR. Amplification was performed with a denaturation of 95° C for 35 s, an annealing temperature of 55° C for

55 s, and an increasing extension period of 72° C for 45 s plus 4 s per cycle over 36 cycles.

Amplified products were digested with the restriction enzymes Hpa II, Cfo I, and Rsa I, separated on 2% agarose gels, stained with ethidium bromide, and digitally photographed for band analysis using the GelCompar II software package (Applied Maths, Inc., Austin TX, 1991). Gel images were processed to eliminate distortion and bending of the gel, and band sizes were calibrated by comparison with a standard DNA ladder. Cluster analysis was performed by GelCompar II using UPGMA with the fuzzy dice procedure, with a band position tolerance of 1%. Results were examined and adjusted by hand as needed.

DNA sequence analysis. For each EMF taxon as defined by distinct RFLP patterns based on cluster analysis, sequences were determined that were used to identify closely related known taxa based on sequence homology. Sequencing of the ITS rRNA gene using the primer pair ITS-1F and ITS-4 was followed by sequencing of approximately 360 bp of the mitochondrial large subunit rRNA gene using primer pair ML5/ML6 described by White et al. (1990) and/or approximately 650 bp of the nuclear large subunit rRNA gene using the primer pair LR0R/LR16 (Moncalvo et al. 2000), as needed to obtain a reliable identification. Closely related sequences were identified using the National Center for Biotechnology Information web-based BLAST search engine (Altschul et al. 1997). PHYLIP version 3.6a3 (Felsenstein 2002) was used to generate neighbor-joining, parsimony, and maximum-likelihood trees to examine the phylogenetic placement of unidentified EMF taxa with reference to published sequences obtained through BLAST searches as well as sequences obtained from EMF taxa fruiting at the study sites. Sequence homology greater than or equal to 98% for the ITS region and 99% for the nLSU rRNA gene were considered sufficient to assign tentative species-level designations to unidentified EMF taxa.

4.2.5 Statistical analyses

EMF taxon abundance, measured as the proportion of root tips for a sample, was divided by total abundance per sample to give relative abundance. The number of taxa observed when sampling a community was adjusted by rarefaction for comparison to communities with a smaller sample size of mycorrhizal root tips. Rarefaction was calculated using the correction proposed by Simberloff (1971) for the method described by Sanders (1968) using a web-based calculator (J. Brzustowski, www2.biology.ualberta.ca / jbrzusto / rarefact.php). This web-based calculator was also used to estimate species richness by the method proposed by Chao (1984). Jackknife estimates of species richness were obtained using the PC-ord software package (McCune and Mefford 1997). The Shannon-Weaver diversity index was calculated as: $H' = -\Sigma p_i \log 10\ p_i$ where p_i were the proportions of individual taxa (Magurran 1988). Simpson's reciprocal diversity index was calculated as 1/D, where $D = \Sigma n(n-1)/N(N-1)$; "n" was the number of root tips for individual taxa and N was the total number of root tips. The Morista-Horn similarity index was calculated as:

$$C_{mh} = \frac{2\ \Sigma a_n b_n}{a_N b_N(\Sigma a_n^2/a_N^2 + \Sigma b_n^2/b_N^2)}$$

where a_n and b_n were the number of root tips for individual taxa from community a and b, and a_N and b_N were the total number of root tips for each community (Magurran 1988).

Comparisons of taxon frequencies were performed using contingency tables (SPSS for Windows 10.0.5, SPSS Inc., Chicago, IL, 1999). Effectiveness of sampling effort was evaluated using 'species area' curves (in this case, 'species-sample unit' curves) calculated by PC-ord. For detrended correspondence analysis (DCA), PC-ord was used to identify outliers and to standardize relative abundance data using the arcsine square-root transformation. The only taxon that qualified as an outlier (>3SD) was "*Rhizopogon* spp." an artificial grouping of *Rhizopogon* mycorrhizas which could not be definitively assigned to a species group. This group was excluded from the ordination analysis, but exclusion did not substantially change the result.

4.3 Results

From 1998 to 2000, 52 taxa were observed on Douglas-fir seedlings and mature trees (Table 4.1). Two taxa within the Atheliaceae could not be resolved to genus and were designated as "Atheliaceae I" and "Atheliaceae II." Sequence homology placed another taxon in the Ascomycota, with an *Aleuria* species as the closest matching sequence but the sequences were not similar enough to place it definitively within this genus, therefore the taxon was designated as "Ascomycota I." Phylogenetic analysis revealed the presence of two clades within the genus *Wilcoxina*, perhaps corresponding to *W. rehmii* and *W. mikolai*, with 70% bootstrap support (data not shown). Because it was not possible to distinguish between the two clades on the basis of RFLPs with the endonucleases used in this study, they were combined as a single taxon and designated as "*Wilcoxina* spp." in this analysis (Table 4.1). Within the genus *Rhizopogon,* while several putative species-level taxa could be distinguished by RFLPs as well as by sequence, some collections could only be identified to the genus level with RFLP analysis, and it was not feasible to sequence each individually due to the large number of samples. Where possible, these were assigned to taxa within the genus *Rhizopogon* based on morphological characters. The remainder are designated as "*Rhizopogon* spp." in Table 4.1. Most of these had RFLP patterns consistent with the "*Rhizopogon* I" found on nursery seedlings which persisted after outplanting, but morphological characters and RFLP patterns could not be used to exclude other *Rhizopogon* species. Within the genera *Russula* and *Tomentella* it was also not possible to distinguish among taxa for all collections, therefore "*Russula* spp.," and "*Tomentella* spp." are included as categories in Table 4.1.

With the exception of the nursery seedlings, all the treatment groups were undersampled, based on species area curves relating the number of taxa observed to the number of samples (Fig.4.1). In particular, the mature tree roots would have required a much higher number of samples to adequately reflect the EMF community, as the species area curve had not begun to level off.

Table 4.1 Relative abundance (%) of EMF on Douglas-fir seedlings and trees.

Taxon	Nursery seedlings[a]	Greenhouse seedlings[b]	>16 m seedlings[c]	<6 m seedlings[d]	Mature trees[e]
Tuber I	59.05	7.06	7.94	5.29	
Rhizopogon I	31.73	10.55	14.89	11.03	
Ascomycota I*	6.33				
Hebeloma album gp.	2.37	5.01	0.66	0.47	
Wilcoxina spp.	0.52	19.67	0.13	0.60	
Tomentella ellisii gp.		27.26	1.65	1.96	
Thelephora terrestris		16.38	2.39	0.52	
Rhizopogon vinicolor var. I		4.79	9.77	6.67	
Rhizopogon spp.		4.77	31.30	20.55	
Cenococcum geophilum		1.94	3.84	2.20	19.48
*Rhizopogon parksii***		1.47	4.60	4.83	
Hymenoscyphus ericae		0.98	0.72	0.03	1.53
Pseudotomentella tristis		0.11	6.57	7.32	11.67
Rhizopogon villosulus			2.49		
Boletus zelleri			2.13	6.20	3.14
Atheliaceae II			1.32	0.08	
Clavulina I			1.11	1.13	8.29
Tomentella sublilacina			0.99	2.78	
Russula spp.			0.98	0.48	5.56
Atheliaceae I			0.91	0.13	
Tomentella stuposa			0.84	1.97	2.01
Pseudotomentella nigra			0.82	0.57	
Tomentellopsis I			0.64		
Tylospora fibrillosa			0.37	2.09	2.14
Amphinema I			0.36	0.06	
Tylospora asterophora			0.36	1.25	
Tomentella spp.			0.36	0.39	
Tomentella II			0.30	0.84	1.33
Piloderma fallax			0.20	0.14	
Melanogaster I			0.19	1.52	
Inocybe II			0.19	0.17	
Russula sphagnophila gp.			0.18	0.08	
Thelephora I			0.09		
Amphinema byssoides			0.06	0.57	
Inocybe sierraensis gp.			0.05		1.14
Cortinarius cinnamomeus			0.03	0.10	0.54
Russula nigricans			0.03	10.98	7.50
Inocybe I			0.02	0.06	
Russula bicolor				1.19	2.06
Lactarius I				1.11	0.64
Russula chloroides				0.98	9.54
Laccaria I				0.98	
Amanita I				0.72	0.72
Sebacina I				0.62	0.78
Truncocolumella citrina				0.61	8.86
Russula amoenipes gp.				0.16	
Inocybe sindonia gp.				0.14	
Peziza I				0.14	
Piloderma byssinum				0.11	4.17
Macowanites americanus				0.10	5.50
Inocybe praetervisa gp.				0.07	
Russula adusta					2.56
Unidentified			0.50	0.02	0.85

Values are means of relative abundance as percent of total mycorrhizal root tips per tree root sample or seedling. For each tree root sample or seedling (except nursery seedlings), a subsample of 100 root tips was examined. Columns sum to 100%. *related to *Aleuria*. **could not be distinguished from *R. vinicolor* II. [a]Nursery-grown seedlings were sampled before planting in spring 1998. [b]Greenhouse seedlings were potted in field soils and sampled 1998-1999. Seedlings were planted in spring 1998 [c]> 16 m or [d]<6 m from mature trees and sampled 1998-2000. [e]Mature tree roots were excavated spring 2000.

4.3.1 EMF of nursery and greenhouse seedlings

Douglas-fir nursery seedlings were mycorrhizal with a total of five EMF taxa at the time of planting, based on a subsample of 8 seedlings with a total of 2612 mycorrhizal root tips. Jackknife estimates, shown in Table 4.2, placed the total taxon richness at 5.9 (1st order) or 6.6 (2nd order), therefore one or two rare taxa may have been missed. The Shannon-Weaver diversity index (H') was 0.41 while the Simpson diversity index (1/D) was 2.12. These low values reflect both the low number of taxa and the low evenness of the EMF community on nursery seedlings. *Tuber* I and *Rhizopogon* I together constituted more than 90% of mycorrhizal root tips (Table 4.1).

Nursery fungi persisted on the planted seedlings both in the greenhouse and in the field, with the exception of an ascomycete related to the genus *Aleuria,* which was not detected after planting (Table 4.1). The nursery dominant *Tuber* sp. decreased in abundance after planting, both under greenhouse and field conditions. It appeared that "*Rhizopogon* I" decreased in abundance after planting both in the greenhouse and at the field sites, although due to the large number of unidentified *Rhizopogon* spp. it was impossible to rule out the possibility that *Rhizopogon* I increased in abundance on field seedlings. Abundance of a species in the *Hebeloma album* group increased in the greenhouse bioassay but decreased for field seedlings. Fungi in the genus *Wilcoxina* were rarely detected on field seedlings, but proliferated dramatically on greenhouse seedlings after planting. This may have been a consequence of the poor mycorrhizal development of greenhouse seedlings, allowing the ectendomycorrhizal *Wilcoxina* to be detected more frequently by molecular methods. On field seedlings, *Wilcoxina* could have been present as a secondary symbiont of tips colonized by ectomycorrhizal taxa forming a well-developed mantle, in which case the molecular signal would likely be obscured during PCR amplification due to the more prevalent DNA from the ectomycorrhizal symbiont.

Table 4.2 Species richness and diversity of Douglas-fir seedling and tree EMF communities.

	Nursery seedlings[a]	Greenhouse seedlings[b]	>16 m seedlings[c]	<6 m seedlings[d]	Mature trees[e]
Samples	8	34	84	84	12
Mycorrhizal root tips	2612	495	3665	4745	339
Taxa observed	5	12	38	47	22
Taxa per sample[f]	3.1 ± 0.1bc	2.0 ± 0.2d	3.3 ± 0.1b	4.1 ± 0.2a	3.6 ± 0.4ab
Estimated species richness					
Chao-1*	ND	ND	38.1	49.0	22.5
Chao-2**	ND	16.5	52.4	59.1	30.6
Jackknife-1*	5.9	14.9	49.9	59.8	32.1
Jackknife-2**	6.6	16.8	56.7	65.8	35.9
Diversity index					
Shannon-Weaver (H')	0.41	0.85	1.09	1.24	1.15
Simpson (1/D)	2.12	5.60	7.22	11.36	11.26

For each tree root sample or seedling (except nursery seedlings), a subsample of 100 root tips was examined. [a]Nursery-grown seedlings were sampled before planting in spring 1998, and all mycorrhizal roots were examined. [b]Greenhouse seedlings were potted in field soils and sampled 1998-1999. Seedlings were planted [c]>16 m or [d]<6 m from mature trees and sampled 1998-2000. [e]Mature tree roots were excavated spring 2000. [f]Values (means ± 1 SE) with different letters are significantly different by Dunnett T3 test (α=0.05). *First-order estimate. **Second-order estimate.

While four of the five nursery EMF taxa persisted after seedlings were potted in soils from the field sites and grown in the greenhouse, eight new taxa colonized the seedlings (Table 4.1). The observed species richness of 12 taxa was estimated to represent an actual species richness of 14.9 taxa (1^{st} order) or 16.8 taxa (2^{nd} order) using jackknife methods (Table 4.2). The Chao-1 and Chao-2 estimates could not be calculated due to the lack of single and double collections in the sample. The Shannon-Weaver and Simpson diversity indices were substantially higher for greenhouse seedlings than for nursery seedlings (Table 4.2). Members of the Thelephoraceae predominated, with 44% of mycorrhizal tips, while *Rhizopogon* spp. were also common, with at least three *Rhizopogon* species together constituting 22% of mycorrhizal tips (Table 4.1). Most of the remaining taxa were ascomycetes, with four taxa making up 30% of mycorrhizal tips, including the *Tuber* sp. from the nursery and the widespread generalist *Cenococcum geophilum* (Table 4.1).

At the time of sampling, 42% of the EMF on root tips of greenhouse seedlings were nursery EMF taxa, meaning that over half the root tips were colonized by taxa obtained from propagules present in the field soils. The EMF taxa that colonized greenhouse bioassay seedlings also colonized field seedlings, but the pattern of relative abundance was different. In general, *Rhizopogon* species were much more abundant on field seedlings compared to greenhouse seedlings, while *Thelephora terrestris* and a species in the *Tomentella ellisii* group were less common on field seedlings. As expected, the taxa colonizing seedlings in the greenhouse treatment were EMF which have been shown to readily colonize seedlings from spores or soil propagules (Bledsoe and Tennyson 1982; Castellano et al. 1985; Berch and Roth 1993; Massicotte et al. 1994; Parlade et al. 1996; Horton et al. 1998b; Baar et al. 1999).

4.3.2 EMF of excavated mature tree roots

A total of 22 EMF taxa were observed on mature tree roots, with estimates of the actual species richness ranging from a low of 22.5 for the Chao-1 estimate to 35.9 for the 2nd order jackknife estimate (Table 4.2). Both observed and estimated taxon richness were substantially higher than for nursery and greenhouse seedlings, but lower than for field seedlings (Table 4.2). The lower species richness was not surprising considering that mature tree roots were collected on only one sample date, while seedling mycorrhizas were collected over a two year period. When compared to field seedling mycorrhizas from spring 2000 only, the EMF community on mature trees had a higher species richness than either <6 m or >16 m seedlings. While an equal number of root tips (1200) were examined for each treatment group (<6 m seedling, >16 m seedling, mature tree), the mature trees had a lower proportion of viable mycorrhizal root tips, and therefore a smaller total sample size, and yet the observed species richness was higher at 22 than the 19 taxa observed for >16 m seedlings and 20 taxa observed for <6 m seedlings. The EMF community on mature trees had greater evenness than that of the <6 m and >16 m seedlings when compared for that sample date only, based on the longer tail of the rank-abundance curve (Fig. 4.2b). The rank-abundance curves for all three treatment groups approximated a log-linear relationship, but the mycorrhizal community on mature trees was more even, as the slope was significantly less than that of the <6 m and >16 m seedlings, based on non-overlap of 95% confidence intervals.

Diversity indices reflected the greater evenness of the EMF communities of the mature trees, in that both indices were higher for mature trees than for >16 m seedlings even over the entire sampling period, despite the substantially lower observed species richness (22 taxa compared to 38 taxa). The Simpson diversity index, which gives more weight to evenness than the Shannon-Weaver index, was much higher for the mature tree than for >16 m seedlings; with 11.26 compared to 7.22, and was close to the value of 11.36 calculated for <6 m seedlings (Table 4.2).

The genus *Rhizopogon* was not detected on mature tree roots (Table 4.1). Instead, mature tree roots were dominated by several taxa in the genus *Russula*, with a combined relative abundance of 27.2 ± 10.4%, and by *Cenococcum geophilum*, with 19.5 ± 5.1%. In addition, *Pseudotomentella tristis*, *Truncocolumella citrina, Clavulina*, and *Macowanites americanus* each had relative abundances greater than 5% (Table 4.1). Of these, *Pseudotomentella tristis* and *Clavulina* were relatively evenly split between <6 m and >16 m field seedlings, while *Truncocolumella citrina*, *Macowanites americanus*, and most of the *Russula* taxa (particularly *R. bicolor* and *R. chloroides*) were found only on <6 m seedlings.

4.3.3 EMF of field seedlings

Seedlings >16 m from the center tree were observed to form mycorrhizas with 38 EMF taxa (Table 4.2). The species area curve was leveling off but had not reached a plateau after sampling 8400 root tips on 84 seedlings from 1998 to 2000 (Fig. 4.1). Estimates placed the actual number of taxa between 38.1 and 56.5 (Table 4.2). The Chao-2 estimate has been shown to be less biased than the jackknife method for samples with low frequencies (Chao 1984; Chao 1987), therefore the estimate of 52.4 ± 8.8 taxa was probably the most reliable. The Shannon-Weaver and Simpson diversity indices, at 1.09 and 7.22, respectively, were higher than for greenhouse seedlings, but lower than for soil cores, mature tree roots, or seedlings planted <6 m of mature trees (Table 4.2). The rank abundance curve was nearly log-linear, with slightly higher abundance of the most dominant taxa (Fig. 4.2a), which were members of the genus *Rhizopogon* (Table 4.1).

On seedlings <6 m from mature trees, 47 EMF taxa were observed (Table 4.2). The same number of root tips were examined for <6 m seedlings as for >16 m seedlings and the species area curve did not reach a plateau (Fig. 4.1). The slope was steeper than for the >16 m seedling EMF community and was similar to the curve for mature tree roots (Fig. 4.1). Rarefaction to a sample size of 3665 root tips (the sample size for >16 m seedlings) yielded an estimate of 46.5 (SD=0.7) EMF taxa on <6 m seedlings, still substantially higher than the number of observed taxa for >16 m seedlings. Estimates of the actual species richness for the EMF community of <6 m seedlings were higher than those for >16 m seedlings, and the Shannon-Weaver diversity index was significantly higher at 1.24 compared to 1.09 for >16 m seedlings ($t_{(7426)} = 12.9$, $p<0.001$) (Table 4.2). Only soil core mycorrhizas had higher diversity indices than <6 m seedlings (Table 4.2). The higher diversity of EMF communities of seedlings <6 m from center trees than of seedlings >16 m from center trees appeared to be due to the presence of a greater number of infrequent taxa, based on the longer tail of the rank-abundance curve (Fig. 4.2a). The EMF community was significantly more even for <6 m seedlings than for >16 m seedlings, as the slope of the rank-abundance curve was significantly steeper for >16 m seedlings (Fig. 4.2a), based on non-overlap of 95% confidence intervals for the slopes.

Five genera were observed to have an abundance greater than 5%, for <6 m and >16 m seedlings considered as a group. Of these, *Rhizopogon* species were more common on >16 m seedlings, with a relative abundance of 63.1 ± 3.0% on >16 m seedlings but only 43.1 ± 3.3% on <6 m seedlings. The nursery taxon *Tuber* sp. was also more common on >16 m seedlings, while *Russula* and *Tomentella* were more common on <6 m seedlings, and the abundance of *Pseudotomentella* species was similar on <6 m and >16 m seedlings. Contingency tables using frequency rather than abundance provided an additional approach to detecting differences between <6 m and >16 m seedling mycorrhizal communities. Six taxa occurred at significantly greater frequencies than expected on <6 m seedlings compared to >16 m seedlings: *Russula nigricans, Tylospora fibrillosa, Tomentella stuposa, Tomentella sublilacina, Boletus zelleri*, and *Melanogaster* sp. (Table 4.3). There were no taxa which occurred at significantly greater frequencies than expected on >16 m seedlings.

The higher diversity and evenness of the mycorrhizal community for <6 m seedlings appeared to be the result of taxa shared with mature trees. Eight of the taxa found on <6 m seedlings but not on >16 m seedlings were also found on roots from the mature tree, including *Russula bicolor*, *R. chloroides* and *Macowanites americanus* (a member of the Russulaceae), *Lactarius* sp., *Amanita* sp., *Truncocolumella citrina*, and *Piloderma byssinum*. In contrast, only one of the taxa found on >16 m seedlings but not on <6 m seedlings was shared with the mature tree, a species in the *Inocybe sierraensis* group (Table 4.1). Taxa found on mature tree roots accounted for 38.8 ± 3.7% of <6 m seedling root tips, but only 17.0 ± 2.6% of >16 m seedling mycorrhizas (Fig. 4.3a). In contrast, >16 m seedlings hosted a greater proportion of EMF observed in the greenhouse bioassay. Greenhouse EMF taxa accounted for 84% of mycorrhizal tips for >16 m seedlings, but only 61% of mycorrhizal tips for <6 m seedlings (Fig. 4.3a). Nursery EMF taxa also appeared to persist more readily on >16 m seedlings (Fig. 4.3a).

The Morista-Horn similarity index, which takes into account the similarity of the distribution of the EMF community, reinforced these findings. <6 m seedlings had mycorrhizal communities with greater similarity than >16 m seedlings to the mycorrhizal communities of the mature tree (Fig. 4.3b). In contrast, >16 m seedlings had greater similarity than <6 m seedlings to mycorrhizal communities of greenhouse bioassay seedlings and nursery seedlings (Fig. 4.3b).

Table 4.3 Results of contingency tables showing EMF taxa occurring at frequencies higher than expected on <6 m seedlings compared to >16 m seedlings.

EMF taxa	Pearson χ^2	*P* value
Russula nigricans	18.389	0.000
All *Russula* spp.	17.111	0.000
Tylospora fibrillosa	4.767	0.029
All *Tylospora* spp.	6.908	0.009
Tomentella stuposa	7.880	0.005
Tomentella sublilacina	5.753	0.034*
All *Tomentella* spp.	13.125	0.000
Boletus zelleri	7.954	0.005
Melanogaster I	6.805	0.009

Seedlings were planted in spring 1998 <6 m or >16 m from mature Douglas-fir trees. Seedlings were excavated in fall and spring from 1998 to 2000. For each seedling, 100 root tips were examined. Pearson χ^2 values are shown for all EMF taxa with *P* values < 0.05. No EMF taxa were present at higher than expected frequencies on >16 m seedlings. **P* value was determined using Fisher's exact test because expected cell values were less than 5.

It was interesting to note that EMF taxa forming epigeous fleshy sporocarps (e.g., Agaricales) were significantly more common for <6 m seedlings than for >16 m seedlings, reflecting a closer similarity to mature tree EMF (Fig. 4.4a). In contrast, taxa forming hypogeous fleshy sporocarps (e.g. *Rhizopogon, Tuber*) were significantly more common for >16 m seedlings than for <6 m seedlings (Fig. 4.4a), while non-truffle forming ascomycetes and resupinates were not significantly different in abundance for <6 m and >16 m seedlings (data not shown). Nevertheless, while proximity to mature trees appeared to have an important effect on mycorrhizal communities based on substantial shifts in similarity indices, the <6 m and >16 m seedling mycorrhizal communities were still more similar to each other (Morista-Horn similarity index = 0.85) than to any other group. Partly due to the high abundance of *Rhizopogon* spp., taxa forming rhizomorphs were significantly more common for <6 m and >16 m seedlings than for mature trees roots and for greenhouse and nursery seedlings (Fig. 4.4b).

4.3.4 Ordination of EMF communities

Ordination of EMF communities using detrended correspondence analysis (DCA) showed clear segregation of field seedling EMF communities from mature tree EMF communities with lower scores on the primary axis, and from greenhouse and nursery seedling EMF communities with higher scores on the primary axis (Fig. 4.5a). The primary axis had an eigenvalue of 0.551 and explained more than half of the variance in the data ($r^2 = 0.56$). The secondary axis had an eigenvalue of 0.262 and explained 16% of the variance in the data; the two axes together explained 72% of the variance.

When analyzed separately, it was clear that <6 m and >16 m seedling EMF communities were only partially segregated along the primary axis, with seedlings <6 m from trees generally scoring higher (Fig. 4.5b). The data were weakly structured. The primary axis had an eigenvalue of 0.412 and explained 33.3% of variation. The secondary axis had an eigenvalue of 0.291 and explained only 4.5% of the variation in the data. Vectors tracing temporal progression from spring to fall and from year to year revealed no clear pattern of change over time (Fig. 4.5b).

4.4 Discussion

4.4.1 EMF of nursery and greenhouse seedlings

The nursery taxon *Rhizopogon* I persisted on greenhouse seedlings potted in field soils. However, *Wilcoxina* spp. matching those from the nursery were able to proliferate to a much greater extent on greenhouse seedlings than on field seedlings. Baar et al. (1999) found both *Rhizopogon* spp. and *Wilcoxina* spp. established readily from resistant soil propagules (i.e., spores) on bishop pine bioassay seedlings. However, *Rhizopogon* spp. were more common on bioassay seedlings than field seedlings while *Wilcoxina* spp. were less common, in contrast to what I observed. It should be noted that my greenhouse treatment differed from a bioassay in that seedlings were previously colonized by nursery EMF taxa. The higher relative abundance of *Rhizopogon* spp. on field seedlings compared to seedlings potted in field soils in my study was intriguing considering the ease with which *Rhizopogon* spp. colonize roots from spores (e.g., Castellano et al. 1985; Parlade et al. 1996; Baar et al. 1999). *Rhizopogon* spp. may have been favored under dry field conditions (Parke et al. 1983). Greenhouse seedlings differed from field seedlings in that they were irrigated and therefore probably experienced little water stress.

Thelephora terrestris was absent from nursery seedlings, but was common on greenhouse seedlings potted in field soils. Because *T. terrestris* is a common greenhouse contaminant, colonization of greenhouse seedlings could have occurred either from spores present in field soils or from local airborne spores. Roth and Berch (1992) found that Douglas-fir nursery seedlings were dominated by *T. terrestris*, however Castellano and Molina (1989) found that presence of *T. terrestris* varied from nursery to nursery. In my study, there was a trend of higher abundance for *T. terrestris* on seedlings >16 m from trees than on seedlings <6 m from trees. Other studies have shown that *T. terrestris* decreased in abundance on seedlings near forest edges compared to seedlings farther into clearcuts (Durall et al. 1998; Kranabetter and Wylie 1998; Kranabetter 1999; Jones et al. 2002). Simard (1997d) observed a six-fold increase in *Thelephora* on trenched Douglas-fir seedlings, implying that forest edge effects on *T. terrestris* abundance may be caused by inability to outcompete EMF taxa linked to mature trees. Kranabetter and Friesen (2002) found that *T. terrestris* was able to replace EMF taxa from the forest when western hemlock seedlings were transplanted into forest openings.

4.4.2 EMF of excavated mature tree roots

The genera *Tylospora* (particularly *Tylospora fibrillosa*), *Tomentella* (particularly *Tomentella sublilacina* and *Tomentella stuposa*), *Russula* (particularly *R. nigricans*), and *Boletus zelleri* occurred more frequently on seedlings <6 m from mature trees than on seedlings >16 m from trees. These EMF taxa were also common on mature tree roots, with the exception of *Tomentella sublilacina*. From this pattern it appears that the predominance of *Tomentella sublilacina* on seedlings <6 m from trees was probably due to differences in the soil environment, while the other four taxa may have colonized seedlings directly from mature tree roots. *Boletus zelleri* was the only EMF taxon forming long rhizomorphs, which would make it a particularly compelling candidate for dependence on hyphal linkages to mature trees.

B. zelleri was more frequent (contingency tables, X^2=10.17, *P*=0.001) and more abundant (single-factor ANOVA, F=5.05, *P*=0.023) on seedlings planted in microsites with buried wood compared to sites lacking buried wood. Other EMF taxa in my study did not appear to be influenced by the amount of buried wood (data not shown). Other studies have observed differences in EMF species composition when wood and soil substrates are compared (Kropp 1982; Goodman and Trofymow 1998a; Smith et al. 2000; Tedersoo et al. 2003). Buried soil wood may be particularly important during periods of drought due to its ability to retain moisture.

4.4.3 EMF of field seedlings

Thirty-eight EMF taxa were observed on Douglas-fir seedlings planted >16 m from mature trees, while 47 taxa occurred on seedlings planted within 6 m of mature trees, using sequence and RFLP analysis. I know of no comparable studies for Douglas-fir. However, these data fall within the range of morphotypes reported on seedlings near forest edges or on isolated trees in studies of other tree species. Naturally regenerated paper birch seedlings near paper birch trees had 47 morphotypes (Kranabetter 1999), while lodgepole pine, white spruce and subalpine fir seedlings planted near forest edges averaged 52 morphotypes per tree species (Kranabetter et al. 1999). Kranabetter and Wylie (1998) found 44 morphotypes on naturally regenerated western hemlock seedlings in four-year-old forest openings near forest edges. Only 25 morphotypes were observed on forest seedlings transplanted into clearcuts, while forest seedlings transplanted into the forest had 38 morphotypes (Kranabetter and Friesen 2002). Simard et al. (1997d) found that trenching reduced the number of morphotypes observed from 17 to 9 for Douglas-fir seedlings growing in mature mixed forests of Douglas-fir and paper birch. By comparison, Roth and Berch (1992) observed 33 morphotypes on Douglas-fir seedlings one year after outplanting into clearcuts on Vancouver Island.

I found that the EMF community was more species rich for seedlings planted <6 m compared to >16 m from mature trees. Diversity indices and rank abundance curves reflected the greater evenness of the EMF community of seedlings <6 m from trees, although the rank abundance curves for each seedling type were very similar by spring of 2000, two years after planting. Studies of seedlings (Hagerman et al. 1999b) and ectomycorrhizal sporocarp production (Sparks 2004) near forest edges provide evidence that 16 m is sufficient to isolate seedlings from mature trees. Nevertheless, I am not able to rule out the possibility that roots or, more likely, mycelial strands connected to mature tree roots could have grown out to reach the >16 m seedlings by the end of the study period.

The relative magnitude of the effect of proximity to isolated mature Douglas-fir trees on EMF diversity of Douglas-fir seedlings was comparable to that observed in several studies of seedlings growing near mature trees (Kranabetter and Wylie 1998; Kranabetter 1999; Hagerman et al. 1999b). The effect was less extreme than the nearly two-fold increase in EMF species richness observed for red oak seedlings growing near oak trees (Dickie et al. 2002a) and for untrenched Douglas-fir seedlings compared to trenched seedlings growing in mixed Douglas-fir forests (Simard et al. 1997d). In contrast, Durall et al. (1998) found only slight decreases in EMF species richness of western hemlock and lodgepole pine seedlings with distance from the forest edge, while Jones et al. (2002) found no differences in species richness with distance for previously colonized Engelmann spruce seedlings planted in mineral soil exposed by mounding. Jones et al. (2003) proposed that forest edge effects on EMF species richness were minimal for previously colonized (i.e., standard nursery) seedlings, but I detected effects of proximity to trees despite the fact that my seedlings were previously colonized. My seedlings retained their nursery EMF taxa after planting, but significant differences in EMF species richness and diversity developed among seedlings <6 m compared to >16 m from residual trees.

Field seedling EMF communities were dominated by *Rhizopogon* spp. which occurred on over half of all mycorrhizal root tips. This appeared to be partly due to the ability of the nursery taxon *Rhizopogon* I to persist and colonize new roots on seedlings after planting. This was not surprising, since Castellano and Trappe (1985) found that inoculated *Rhizopogon vinicolor* persisted and colonized new roots on Douglas-fir seedlings after outplanting in clearcuts. For the genus as a whole, *Rhizopogon* spp. were present on 63% of root tips for seedlings >16 m from mature trees but only on 43% for seedlings <6 m from trees. The dominance of *Rhizopogon* spp. was responsible for the significantly higher proportion of taxa forming rhizomorphs on field seedlings than on nursery and greenhouse seedlings and on mature roots. The long rhizomorphs produced by *Rhizopogon* are thought to be adapted for long-distance transport (e.g., Agerer 2001), but may also play a role in competing with other EMF taxa for the colonization of new roots. Simard et al. (1997d) found that *Rhizopogon vinicolor* was 20 times more abundant on untrenched Douglas-fir seedlings than on trenched seedlings, and suggested that *R. vinicolor* benefited from hyphal linkages to mature trees. This did not appear to be the case in my study, since seedlings >16 m from trees had higher abundance of *Rhizopogon* spp. than seedlings <6 m from trees.

Several of the EMF taxa found more frequently on seedlings <6 m from trees have been reported to be favored by high soil nitrogen conditions, including *Tomentella sublilacina* and *Tylospora fibrillosa* (Taylor et al. 2000; Lilleskov et al. 2002), and *Russula* spp. (Avis et al. 2003). Nevertheless, it seems unlikely that nitrogen would be more available near trees. In Douglas-fir forests near my study sites, Barg and Edmonds (1999) found no differences in soil nitrogen dynamics at 6 m compared to 1 m from isolated mature trees. Nitrogen is often more available in the first few years after forest harvesting. Parsons et al. (1994a) detected increases in soil nitrate in experimentally created gaps of 15 or more trees in lodgepole pine forests. Also, only some of the EMF taxa occurring more frequently <6 m from trees were associated with high nitrogen substrates. For example, *Tomentella stuposa* was reported by Tedersoo et al. (2003) to predominate in coarse woody debris, which usually has a high carbon to nitrogen ratio.

4.4.4 Ordination of EMF communities

EMF communities of seedlings <6 m from and >16 m from trees were not entirely segregated when all EMF communities were included in the ordination. Nevertheless, EMF communities of seedlings <6 m from trees had generally lower scores on the primary axis, closer to the scores for mature tree roots (Fig. 4.5a). The secondary axis appeared to be associated with site differences for all field samples. EMF of mature trees had the greatest separation by site along axis 2, with low scores for Imagine, high scores for Green River, and intermediate scores for Beatles. EMF of seedlings <6 m from mature trees were less separated by site, but followed the same pattern. EMF of seedlings >16 m from mature trees were not as strongly separated by site, and the position of Beatles and Imagine were reversed compared to seedlings <6 m from trees. Greenhouse seedling EMF clustered tightly together, indicating that the site of origin of the soil in which the seedling was planted had relatively little effect on the EMF community.

Ordination of only the field seedling EMF communities revealed an effect of proximity to mature trees which was related to the primary axis. When proximity was coded as a quantitative factor (<6 m = 1, >16 m = 0), the treatment effect accounted for 25.5% of variation along axis 1 ($r = 0.505$). The secondary axis again appeared to be related to site. Imagine was associated with high values, Green River was intermediate (but highly variable) and Beatles was associated with low values along axis 2. It was notable that samples from seedlings <6 m from trees at Green River assorted closely with seedlings >16 m from trees at the same site. This was responsible for most of the overlap between EMF communities of the two groups of seedlings. In general, differences among sites were greater than differences between seedlings <6 m compared to >16 m from mature trees at each site.

Seedlings <6 m from mature trees had a greater proportion of mature tree taxa, and the EMF community was more similar to that of mature trees. Seedlings >16 m from trees had a greater proportion of taxa found on greenhouse seedlings, and the EMF community was more similar to that of greenhouse seedlings and nursery seedlings. Ordination reflected these patterns. The EMF community from seedlings <6 m from mature trees was associated with the mature tree communities. The EMF community for seedlings >16 m from trees was more closely associated with communities of greenhouse and nursery seedlings. It is not clear whether this similarity is a result of the direct influence of mature trees on colonization of seedlings, or a secondary effect of trees on the soil environment which facilitates the colonization or persistence of taxa associated with mature forest soils.

4.5 Conclusions

Residual trees play a dual role in recently harvested forests. First, by retaining a unique EMF community on their root systems, and second, by influencing the species composition and enhancing the diversity of EMF communities on seedlings adjacent to the trees. I have also shown for these seedlings that proximity to trees increases colonization levels and root to shoot ratios, while generally inhibiting stem growth (Chapter 3). The long term impact of residual trees on seedling survival and growth is difficult to predict, but it appears likely that residual trees would have lasting impacts on mycorrhizal diversity at the landscape level (i.e., at wider spatial scales) as stands recover from the effects of harvesting.

Differences in the mycorrhizal communities of seedlings as a result of proximity to mature trees could have important functional consequences not just for host plants but for the ecosystem as a whole as harvested forests regenerate. High mycorrhizal diversity has been proposed to increase plant productivity (van der Heijden et al. 1998; Jonsson et al. 2001) and potentially influence plant responses to environmental fluctuations or disturbance (Bledsoe 1986; Jones et al. 2003). In addition to promoting EMF biodiversity, presence of residual trees influenced the species composition of mycorrhizal communities, which could impact ecosystem-level processes. For example, taxa producing fleshy epigeous sporocarps were significantly more abundant on seedlings <6 m from trees. If the balance between epigeous and hypogeous sporocarp production were to be influenced over the longer term, this could have important consequences for mycophagous animals.

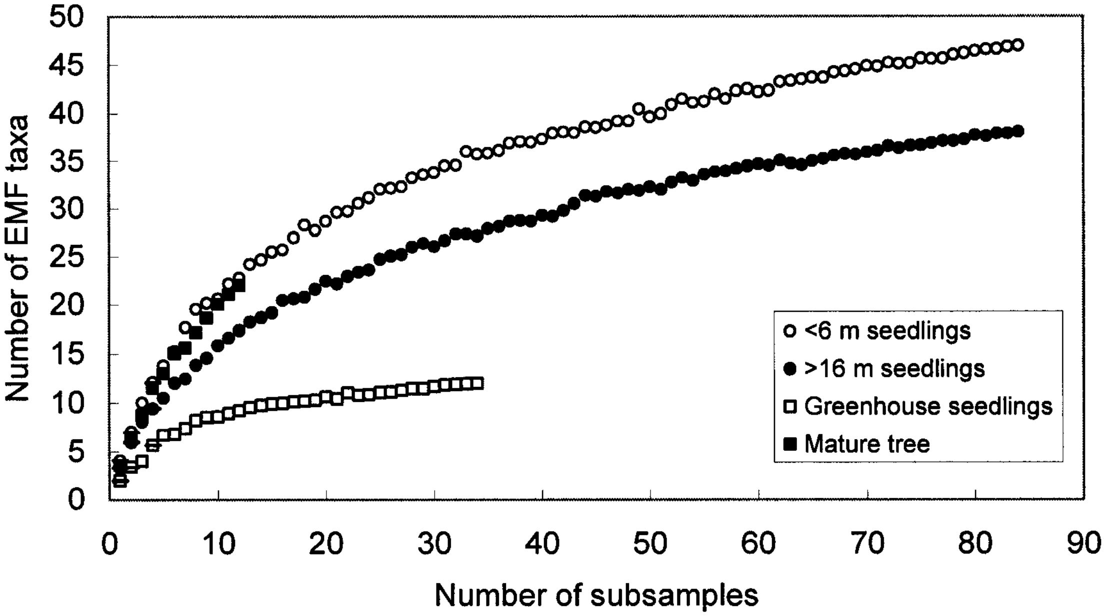

Fig. 4.1 'Species-area' curves showing increase in number of observed EMF taxa with increased sampling effort of Douglas-fir seedlings and trees.
Seedlings were planted in spring 1998 <6 m or >16 m from mature trees and sampled 1998 to 2000, greenhouse seedlings were potted in field soils in spring of 1998 and sampled from 1998 to1999, and mature tree roots were excavated spring 2000. Each subsample consisted of 100 root tips.

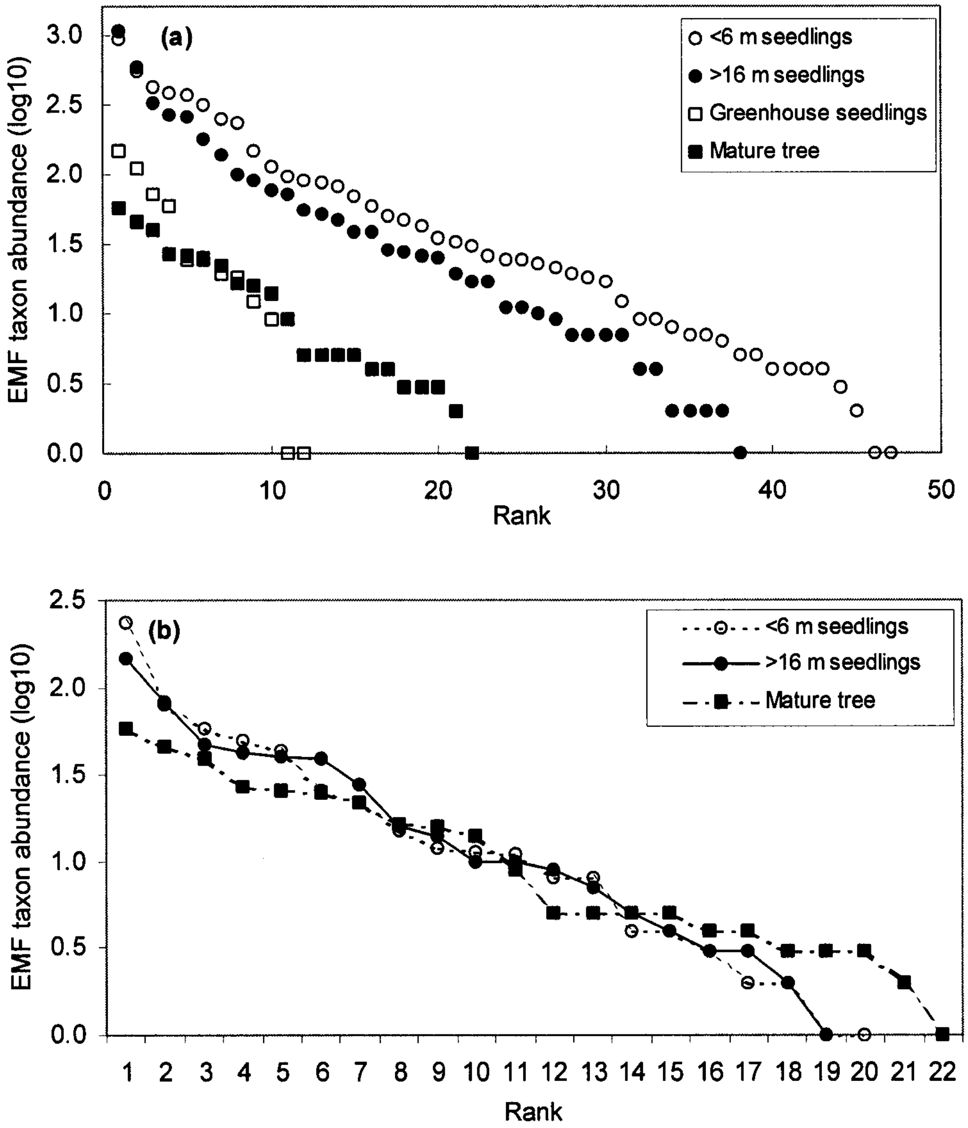

Fig. 4.2 Rank-abundance curves for EMF taxa of Douglas-fir seedlings and trees. Slopes (B) of best fit lines ($\log_{10}$(abundance) rank^{-1} are given for each. (a) Seedlings were planted in spring 1998 <6 m (B=-0.054) or >16 m (B=-0.066) from mature trees and sampled 1998-2000, greenhouse seedlings (B=-0.183) were potted in field soils spring 1998 and sampled from 1998 to 1999, and mature tree roots (B=-0.074) were excavated spring 2000. (b) EMF rank abundance spring 2000 only for <6 m seedlings (B=-0.109), >16 m seedlings (B=-0.103) and mature trees (B=-0.074).

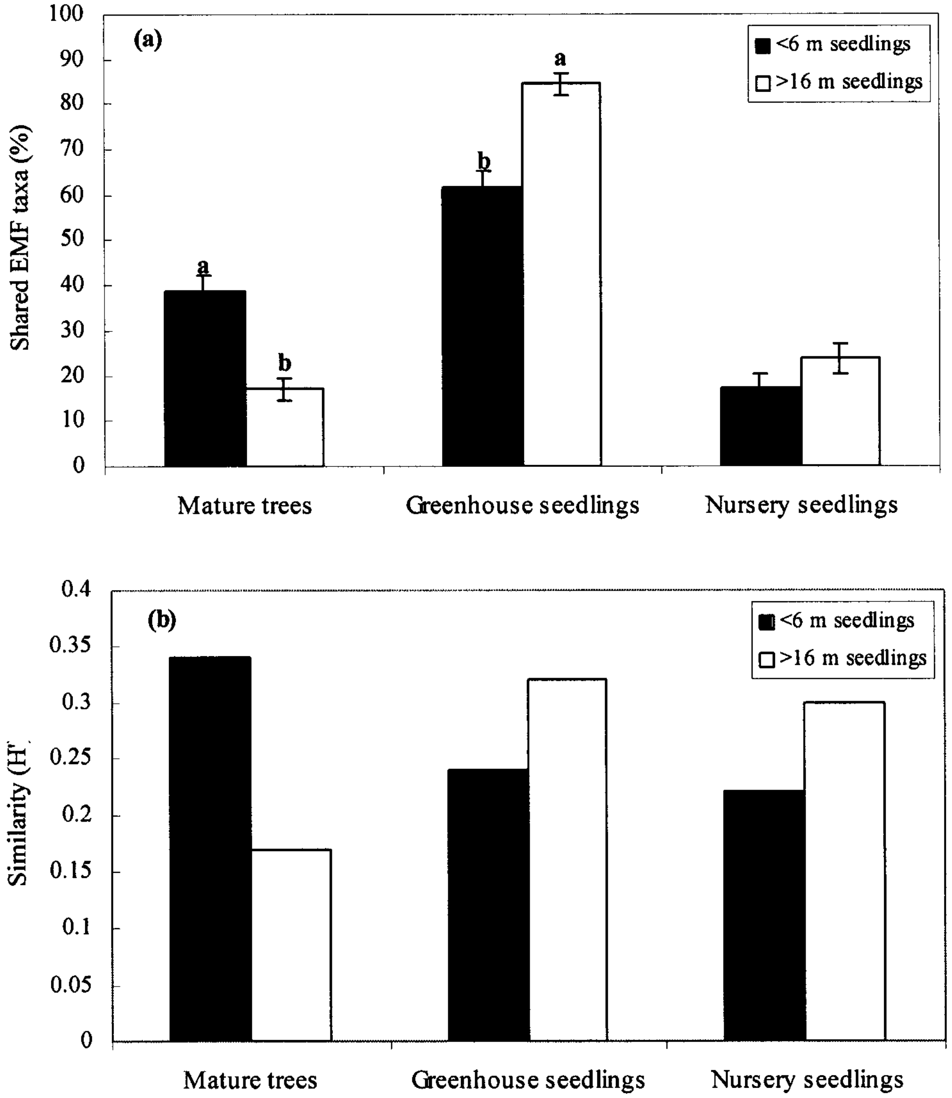

Fig. 4.3 Similarity of EMF communities of Douglas-fir seedlings <6 m and >16 m from trees to EMF of mature trees and greenhouse and nursery seedlings.
(a) Proportion of EMF taxa shared with mature trees, greenhouse seedlings potted in field soils, and standard bare-root nursery seedlings. Values are means of relative abundance ± SE of shared EMF taxa. Groups (<6 m, >16 m) with different letters are significantly different by ANOVA ($\alpha=0.05$). (b) Morista-Horn similarity index (H') comparing <6 m seedlings to >16 m seedlings based on similarity to mature trees, greenhouse, and nursery seedlings.

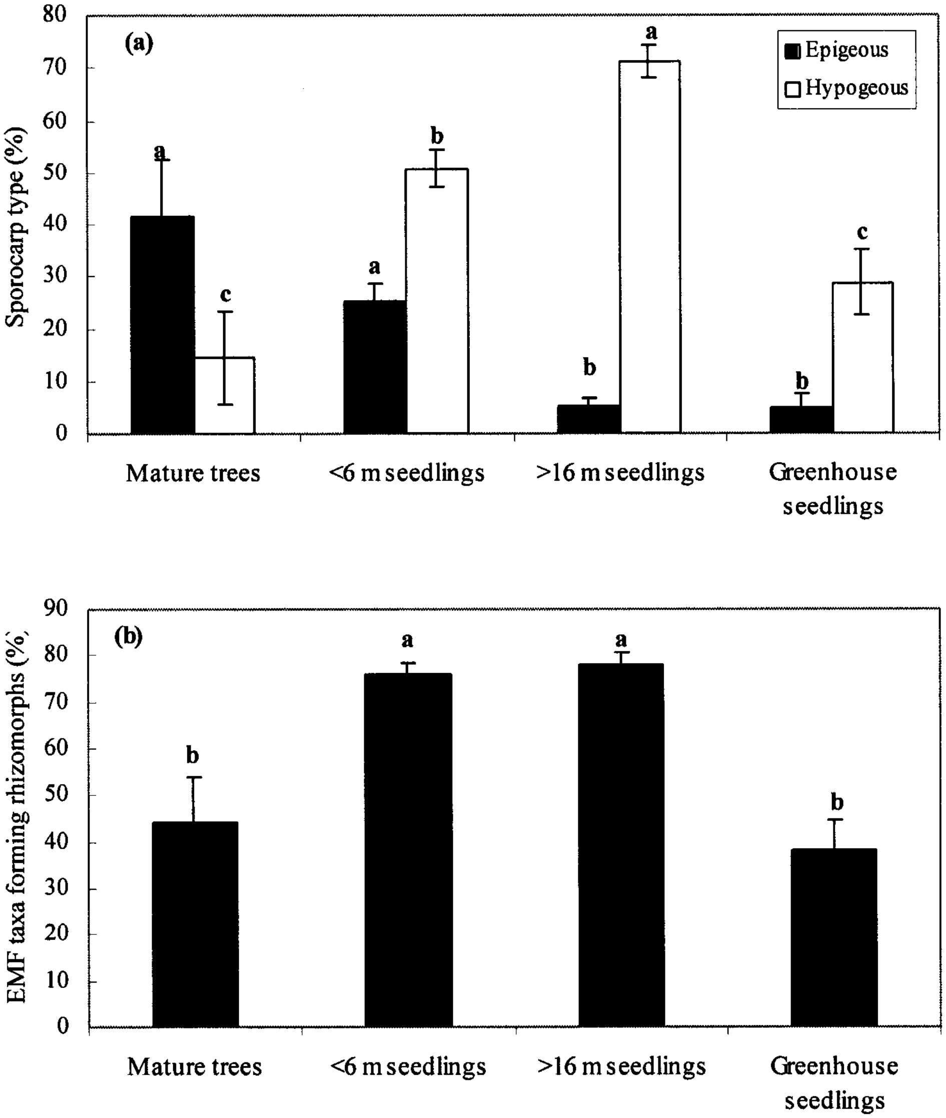

Fig. 4.4 Percent relative abundance of EMF functional groups from Douglas-fir seedlings and trees.

(a) Epigeous includes taxa forming fleshy aboveground sporocarps (e.g., Agaricales), hypogeous includes taxa forming fleshy belowground sporocarps (e.g., *Tuber*, *Rhizopogon*). Sporocarp categories do not sum to 100% because resupinates and non-truffle-forming ascomycota are not shown. (b) Percent relative abundance of EMF taxa forming rhizomorphs. Seedlings were planted <6 m or >16 m from mature trees. Greenhouse seedlings were potted in field soils. Values are means ± 1 SE. EMF communities with different letters are significantly different by Tukey's HSD (α=0.05).

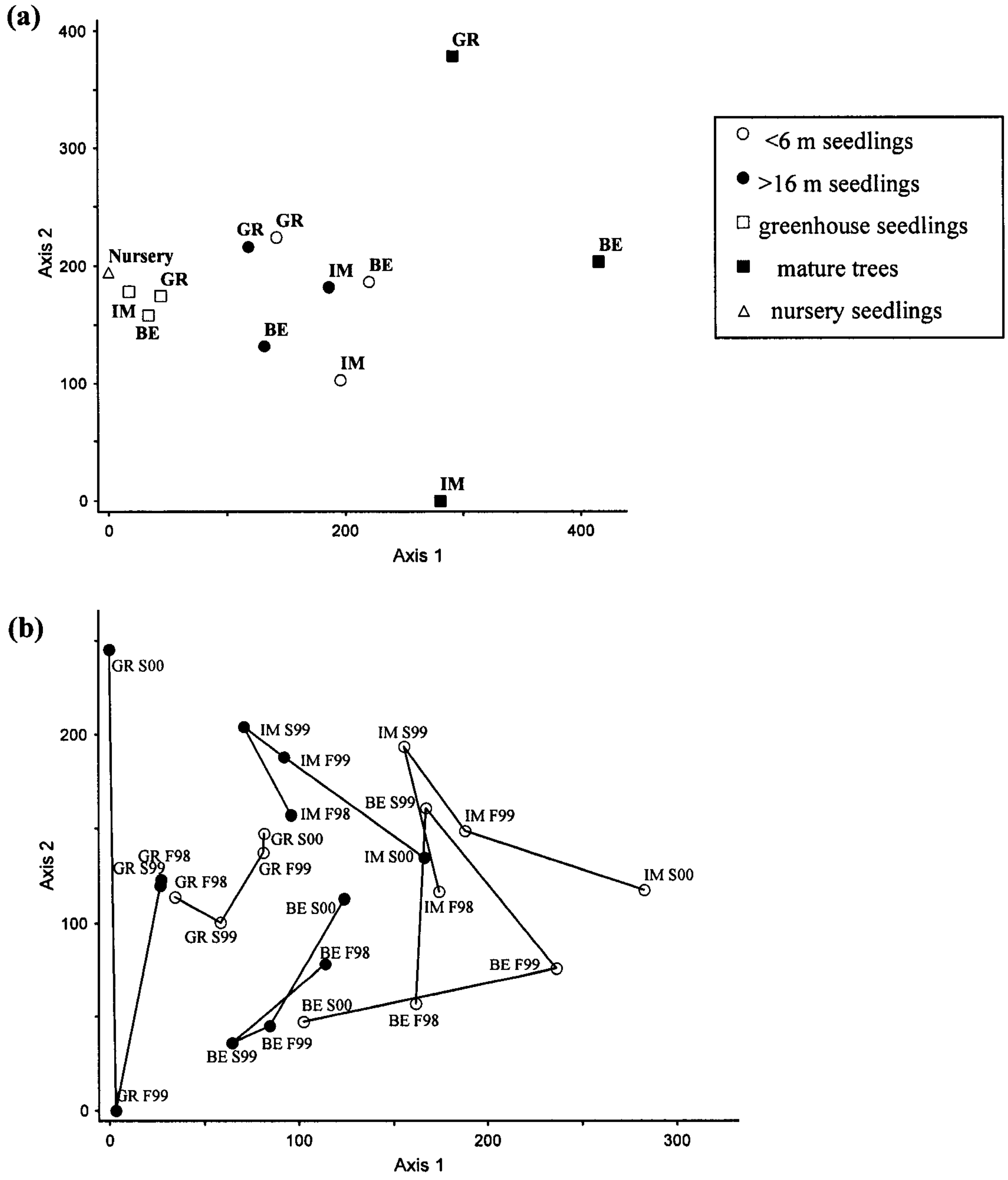

Fig. 4.5 Detrended correspondence analysis (DCA) of EMF communities on Douglas-fir seedlings and trees.

Groups consist of seedlings planted <6 m and >16 m from mature trees, greenhouse seedlings, mature tree roots, and nursery seedlings. DCA was used to analyze arcsin square-root transformed relative abundance of EMF taxa. Outliers (>3 SD) were excluded from the analysis. (a) All groups, with rare taxa downweighted. Samples are labeled by site (GR=Green River, IM=Imagine, BE=Beatles). (b) <6 m and >16 m seedlings only. Plots are labeled by site and season and year of sampling (F=fall, S=spring). Lines are vectors showing temporal progression for each plot.

Chapter 5. Mycorrhizal Fungus Communities of Residual Douglas-fir Trees: Changes with Time after Harvesting

5.1 Introduction

Some ectomycorrhizal fungi (EMF) taxa in conifer forests are associated with young forests, some taxa are restricted to mature forests, while others are generalists. The abundance of these groups changes as stands mature after disturbance (Visser 1995; Byrd et al. 2000; Shaw et al. 2003). The controls on EMF succession are not well understood. EMF taxa associated with mature forests can be dependant on the photosynthate supplied by hyphal linkages to mature trees (Fleming 1984), but changes in the soil environment after disturbance may also play a role. Abundance (Harvey et al. 1978; van der Heijden and Vosatka 1999) or activity (Griffiths et al. 1990) of EMF taxa may also change from season to season, but few studies have examined this.

Forest harvesting modifies physical, chemical, and biological components of the soil environment, which may select for fungi better suited for the altered environment (Jones et al. 2003). Kranabetter and Friesen (2002) demonstrated that some EMF taxa were lost from forest seedlings transplanted into gaps, suggesting that changes in the soil environment could have excluded EMF taxa adapted to forest conditions, at least for seedlings. For mature trees, harvesting impacts on EMF communities have been observed near forest-clearcut edges and in recently thinned forests. In one-year-old clearcuts, Hagerman et al. (1999a) found that diversity of ectomycorrhizas in soil cores was lower in the clearcut than in the forest, even within 2 m of the forest edge (i.e., within the rooting zone), implying that EMF communities may be impacted by harvesting even when their host tree survives. These changes could be due to harvesting impacts on the belowground environment. Sparks (2004) documented changes in soil moisture and decomposition rates at forest edges, related to patterns of sporocarp production for various ectomycorrhizal taxa. Impacts of forest thinning are less clear, however. EMF communities responded to forest thinning with significant decreases in species richness in late-successional Douglas-fir forests in Oregon, but species richness did not decline within the dripline of residual trees (Stockdale 2000).

Douglas-fir forest management in the Pacific Northwest has been transformed in the last decade due to concerns about declining biodiversity. A management strategy that has been widely adopted in place of clearcutting is the retention of some proportion of living trees when harvesting, often referred to as green-tree-retention or residual forestry (Franklin 1993). Residual trees have been proposed to allow for the persistence of forest-dependent species while the young forest regenerates. This might be of particular importance for mycorrhizal fungi, but the effectiveness of residual trees in harboring forest-associated EMF taxa has not been evaluated in the Pacific Northwest. The impact of harvesting on the belowground environment near isolated residual trees would likely be greater than near intact forest edges or in thinned forests, but it is not known how EMF taxa associated with isolated residual trees respond to the environmental changes resulting from harvesting.

The objective of this study was to assess changes of EMF communities in harvested stands with residual Douglas-fir trees. Specifically, the goal was to determine (i) annual changes in the EMF community after harvesting, (ii) changes from spring to fall in EMF communities, and (iii) how soil characteristics are related to the distribution of EMF taxa.

5.2 Materials and methods

5.2.1 Site characteristics

This study took place in recently harvested second growth Douglas-fir forests in the foothills of the western Cascade mountain range in Washington state, USA, approximately 50 km from the city of Seattle. Two sites, "Beatles" (488 m elevation, GPS coordinates 47° 20.970' N, 121° 49.895' W) and "Imagine" (457 m, 47° 23.956' N, 121° 48.938' W) were on public land managed by the City of Seattle Cedar River watershed while one site, "Green River" (808 m, 47° 18.719'N, 121° 42.609' W) was owned and managed by Plum Creek Timber Company. The sites were between 7 and 13 km from each other. Green River was harvested in 1996, Imagine was harvested in 1994, and Beatles was harvested in 1992. Sites were selected based on the presence of mature isolated residual Douglas-fir trees. Site characteristics are summarized in Chapter 3 in Table 3.1.

5.2.2 Sampling of soil cores

From 1998 to 1999, during the growing season, monthly soil cores were obtained at each of the three sites. In preliminary sampling, mycorrhizas were inactive during the dry summer months, therefore cores were not collected in July and August of 1998. Sample points were chosen at random distances from 2 to 6 m along four random bearings from the base of each center tree. A bulk density sampler was used to obtain two soil cores (10 cm diameter x 20 cm depth) from within 50 cm of each sample point, which were aggregated to reduce variability. A 10 g subsample was taken from each aggregated sample for soil moisture and pH determination. Soils were pre-weighed and dried to constant weight at 105° C. Soil percent moisture was calculated as the change in weight divided by the dry weight of the soil sample. Soil pH was determined by mixing 10 g of soil with 25 ml of distilled water, then measuring the pH after settling using a pH probe.

Soil samples were washed in a root elutriator (a "root jacuzzi"), in 0.1 M NaPyrophosphate ($Na_4P_2O_7$, Fisher Scientific Inc.). All mycorrhizal Douglas-fir root tips within each composite soil sample were removed for further analysis after examination of roots using an 8-40X dissecting microscope. Non-Douglas-fir roots were excluded from analysis on the basis of gross morphological characters, but western hemlock roots were difficult to distinguish and some may have been accidentally included in the sample. Although sample points were adjusted to avoid sampling directly under seedlings to minimize sampling of seedling roots, it was not possible to differentiate between mature tree roots and Douglas-fir seedling roots in soil cores.

5.2.3 Morphological and molecular identification of mycorrhizal fungi

Mycorrhizal root tips were identified by examination using a dissecting microscope. Root tips lacking a visible mantle were presumed to be mycorrhizal and sampled for molecular analysis unless abundant root hairs were present. Root tips were considered inactive or necrotic and were not sampled if the entire root tip was shrunken and dessicated or if the root cortex was partially or completely decayed or absent, leaving only the stele. Mycorrhizal root tips were sorted into broadly defined morphotypes based on morphological characteristics (Ingleby et al. 1990; Agerer 1991; Goodman et al. 2000). Root tips from each morphotype for each root sample were counted and lyophilized to prepare for long-term storage and/or DNA extraction. DNA was extracted from mycorrhizal root tips using the method described by Gardes and Bruns (1993) with the modifications described in Chapter 3.

For every morphotype from each soil sample, a root tip was selected at random for RFLP analysis using the primers ITS-1F (Gardes and Bruns 1993) and ITS-4 (White et al. 1990) and the restriction enzymes Hpa II, Cfo I, and Rsa I. As needed, additional root tips were analyzed when PCR was unsuccessful for the first root tip. Banding patterns were analyzed with the GelCompar II software package (Applied Maths, Inc., Austin TX, 1991) using UPGMA cluster analysis with the fuzzy dice procedure and a band position tolerance of 1%. Results were examined and adjusted by hand as needed.

For each RFLP-type, the sequence of the ITS rRNA gene was determined using the primer pair ITS-1F and ITS-4. Closely related sequences were identified using the National Center for Biotechnology Information web-based BLAST search engine (Altschul et al. 1997). PHYLIP version 3.6a3 (Felsenstein 2002) was used to generate neighbor-joining, parsimony, and maximum-likelihood trees to examine the phylogenetic placement of unidentified fungal taxa with reference to published sequences obtained through BLAST searches as well as sequences obtained from fungal sporocarps collected at the study sites. When the ITS region could not be successfully analyzed, sequences were determined for a less variable 360 bp segment of the mitochondrial large subunit rRNA gene using the primer pair ML5/ML6 described by White et al. (1990). Sequences were also determined for a 650 bp segment of the nuclear large subunit rRNA gene using the primer pair LR0R/LR16 (Moncalvo et al. 2000), as needed to confirm identifications. Sequence homologies of 98% or greater for the ITS region and 99% or greater for the nLSU rRNA gene were considered sufficient to assign tentative species-level designations to unidentified fungal taxa.

5.2.4 Statistical analyses

EMF taxon abundance, measured as the proportion of root tips for a sample, was divided by total abundance per sample to give relative abundance. For each site, rarefaction was used to adjust for year to year changes in sample size of mycorrhizal root tips. Rarefaction was calculated using the correction proposed by Simberloff (1971) for the method described by Sanders (1968) using a web-based calculator (J. Brzustowski, www2.biology.ualberta.ca/jbrzusto/rarefact.php). This web-based calculator was also used to estimate species richness by the method proposed by Chao (1984). Species-area curves and jackknife estimates of species richness were obtained using the PC-ord software package (McCune and Mefford 1997). The Shannon-Weaver diversity index was calculated as:

$$H' = -\Sigma p_i \log_{10} p_i$$

where p_i were the proportions of individual taxa (Magurran 1988). Simpson's reciprocal diversity index was calculated as 1/D, where

$$D = \Sigma n(n-1)/N(N-1)$$

with "n" the number of root tips for individual taxa and "N" the total number of root tips.

For ordination, relative abundance data was standardized using the arcsine square-root transformation and detrended correspondence analysis (DCA) was performed using the PC-ord software package (McCune and Mefford 1997). Chi-square tests for contingency tables, ANOVA, general linear modeling, and other standard statistical tests were performed using the SPSS software package (SPSS for Windows 10.0.5, SPSS Inc., Chicago, IL, 1999) with $\alpha=0.05$ unless otherwise stated.

5.3 Results

The combination of RFLP analysis and sequence analysis of multiple ribosomal genes was generally quite effective at identifying closely matched taxa from sequence databases (Table 5.1). There remained some limitations to the method, however. Two taxa within the Atheliaceae, designated as “Atheliaceae I” and “Atheliaceae II” could not be resolved to genus. For the genera *Rhizopogon, Russula, Tomentella*, and *Wilcoxina*, phylogenetic analyses of sequence data revealed the presence of multiple clades distinct enough to belong to separate species (data not shown). RFLP analysis with the endonucleases used in this study could not always distinguish among these clades. This was particularly common for mycorrhizas from the genus *Rhizopogon*. Collections which could not be assigned to defined species groups within these genera were assigned to artificial clades and designated as “spp.” (for example, *Rhizopogon* spp.). An additional factor was the small number of viable root tips in the typical soil core, which resulted in many single collections for each morphotype in each core. Poorly developed (i.e., recently colonized) mycorrhizas presented additional challenges since PCR amplification was problematic, often resulting in faint RFLP bands which were difficult to interpret with confidence. Nevertheless, nearly 90% of fungal sequences from collected root tips could be assigned to a genus, and the majority could be tentatively assigned to a species.

Conifer roots in general, and Douglas-fir roots in particular, were not abundant in soil cores. Instead, many soil cores contained dense mats of roots from grasses, ferns, or shrubs, with few or no conifer roots present. When Douglas-fir roots were present, they often lacked active mycorrhizal root tips; instead, root tips were frequently necrotic. Mycorrhizal Douglas-fir roots averaged only 3.1 ± 0.7 root tips per liter of soil volume, and were absent from 44% of aggregated soil cores. The proportion of empty soil cores was highest at Imagine (58%), slightly lower at Green River (50%) and substantially

Table 5.1 Relative abundance (percent) of EMF taxa in soil cores near isolated Douglas-fir trees.

	Green River (harvested 1996)		Imagine (harvested 1994)		Beatles (harvested 1992)	
EMF taxon	1998[a]	1999	1998	1999	1998	1999
Pseudotomentella tristis	28.9	12.2			7.3	4.8
Tomentella viridula	27.8	7.3				
Tuber I	14.4				4.2	1.3
Clavulina	7.8	20.1		9.8		0.7
Rhizopogon spp.	5.6				20.3	2.9
Cenococcum geophilum	4.4	3.0		5.7	11.5	2.4
Russula bicolor	3.3	8.5			4.2	2.4
Tomentella II	1.1					
Macowanites americanus		28.0				
Rhizopogon vinicolor I		6.7		0.8		13.2
Tomentella spp.		6.1				3.5
Piloderma byssinum		4.3			3.6	3.3
Russula spp.		2.4	20.0	1.6	3.1	7.0
Piloderma fallax		0.6				
Tomentella stuposa		0.6		1.6		
Atheliaceae II			24.0			
Boletus zelleri			20.0	5.7	0.5	3.5
Russula nigricans				52.8	5.2	9.4
Tylospora asterophora				13.0		
Russula sphagnophila				0.8		
*Rhizopogon parksii**					7.8	19.3
Thelephora terrestris					7.8	4.2
Wilcoxina spp.					7.3	
Laccaria					6.3	0.9
Tylospora fibrillosa					3.1	0.7
Inocybe praetervisa gp.					1.0	0.7
Russula xerampelina						5.7
Tomentella sublilacina						1.8
Amphinema I						0.9
Tomentella citrina						0.7
Atheliaceae I						0.2
Thelephora I						0.2
Unidentified	6.7	0	36.0	8.1	6.8	10.3

Values are means of relative abundance as percent of total mycorrhizal root tips per soil core. Columns sum to 100%. Monthly growing season soil cores were taken to a depth of 20 cm and sampled five times monthly during the growing season in 1998 and 1999. [a]April 1998 core could not be obtained due to snow conditions. *could not be distinguished from *R. vinicolor* II.

lower at Beatles (25%). Active mycorrhizal root tip densities were lowest at Imagine and highest at Beatles (Table 5.2). Densities increased from 1998 to 1999 at each of the three sites (Table 5.2), although the difference was only significant at Beatles (single-factor ANOVA, F=5.46, *P*=0.048). Western hemlock roots were common at Imagine, found occasionally at Beatles, but were not found in soil cores at Green River. The density of mycorrhizal root tips was not significantly correlated to soil moisture or pH when controlling for site differences. Due to the high proportion of empty soil cores, mycorrhizal abundance data from replicate soil cores (n=4) were combined in analyses to minimize variability.

5.3.1 EMF taxa

From 1998 to 1999, 33 EMF taxa were observed in soil cores taken near mature Douglas-fir trees. In total, 1139 mycorrhizal root tips were examined and 212 were selected for DNA extraction and RFLP analysis. Six taxa were present in soil cores at greater than 5% relative abundance for all sites and dates considered together. In order of abundance, they were *Russula nigricans, Boletus zelleri*, *Rhizopogon parksii, Clavulina* I, *Rhizopogon vinicolor* and *Cenococcum geophilum* (Table 5.1). Members of the genus *Russula* were dominant with 23% of all mycorrhizal root tips. Members of the genus *Rhizopogon* were also abundant, found on 17% of root tips. Members of the family Thelephoraceae (e.g., *Thelephora, Tomentella, Pseudotomentella*) constituted 15% of root tips while 8% of root tips were colonized by members of the Atheliaceae (e.g., *Tylospora, Piloderma*). Over half of the root tips in soil cores (54.9 ± 5.2%) were colonized by fungal taxa that produce rhizomorphs. Fungal taxa were relatively evenly split between those forming epigeous sporocarps (39.9 ± 5.1%), those forming hypogeous sporocarps (22.2 ± 4.2%), and resupinates and others that do not produce conspicuous sporocarps (28.4 ± 4.7%). The abundance of these functional groups did not change significantly from 1998 to 1999 (data not shown).

Table 5.2 Soil moisture and pH, species richness and diversity of EMF communities in soil cores near isolated Douglas-fir trees.

	Green River		Imagine		Beatles	
	1998	1999	1998	1999	1998	1999
Samples	4	5	5	5	5	5
Soil pH[1]	4.4 ± 0.2	4.0 ± 0.2	4.0 ± 0.04	3.7 ± 0.1	4.2 ± 0.2	3.8 ± 0.2
Soil % moisture*	83 ± 24	71 ± 7	85 ± 16	97 ± 16	86 ± 15	99 ± 4
EMF root tips (total #)	90	164	25	123	192	545
EMF density (l^{-1})*[a]	1.8 ± 1.1	2.6 ± 0.9	0.40 ± 0.12	2.0 ± 1.1	3.1 ± 1.7	8.7 ± 1.7
EMF taxa per sample*	2.5 ± 1.0	4.2 ± 1.1	1.2 ± 0.4	2.6 ± 0.9	4.2 ± 1.3	7.4 ± 1.0
EMF taxa observed	9	12	4	10	16	24
Rarefaction to 1998**[b]		11.0 ± 0.7		6.0 ± 1.0		21.0 ± 1.1
Estimated species richness						
Chao 1st order**	ND	ND	ND	11 ± 1.6	16 ± 1.1	ND
Chao 2nd order**	41 ± 23	14 ± 2	5 ± 2	18 ± 7	40 ± 14	56 ± 17
Jackknife 1st order	15.0	16.0	5.6	15.6	25.6	36.8
Jackknife 2nd order	18.7	16.3	5.9	18.5	31.4	44.6
Diversity index						
Shannon-Weaver H'	0.80	0.90	0.59	0.68	1.10	1.16
Simpson 1/D	5.28	6.53	4.23	3.19	11.04	11.02

Each sample consisted of the combination of 4 replicates of 2 aggregated 10 cm diameter x 20 cm soil cores for a total soil volume of 13 l. ND, estimate could not be determined. *Values are means ± 1 SE. **Values are estimates ± 1 SD. [a]Number of viable EMF root tips per liter of soil volume. [b]Rarefaction to adjust number of EMF taxa observed in 1999 for the 1998 sample size of viable EMF root tips.

5.3.2 EMF diversity and community structure

After two years of monthly sampling, a plot of the mean number of taxa observed with each additional monthly sample during the growing season (a so-called 'species-area' curve) had yet to approach a maximum at any of the sites (Fig. 5.1). Additional sampling would have likely yielded an increase in the number of taxa observed. Due to the relatively low number of mycorrhizal root tips in each soil core, it was not clear whether the increase in taxa with additional sampling was due to recruitment of new fungal taxa to roots over time, or merely due to observations of taxa which had been missed in earlier sampling. More new taxa were encountered at Beatles with each additional sample than at Green River, and at Green River than at Imagine (Fig. 5.1). Rank abundance for the three sites followed the same trend (Fig. 5.2), with a steeper (negative) slope of the best-fit line for Imagine than for Green River, and a steeper slope for Green River than for Beatles, based on non-overlap of 95% confidence intervals. While the abundance of mycorrhizal root tips in soil cores increased from 1998 to 1999, the shape of the rank-abundance curve was similar from year to year (Fig. 5.2) and the slope of the rank abundance best-fit lines did not change significantly from 1998 to 1999 for any of the sites. In general, the rank abundance curves were close to log-linear, with a right-shift in the middle due to the relatively large number of moderately abundant taxa.

The number of EMF taxa per sample and the total number of taxa observed increased from 1998 to 1999, however this corresponded to an increase in the number of mycorrhizal root tips per soil core (Table 5.2). When rarefaction was used to adjust for the smaller sample size in 1998 at each site, the estimate remained substantially higher for 1999, indicating that the difference was not solely caused by changes in abundance from year to year (Table 5.2). Chao and jackknife estimates of species richness were also lower for 1998 at Imagine and Beatles, but not at Green River, although several of the estimates had large standard deviations due to the small sample size (Table 5.2). While the Shannon-Weaver diversity index (H') was higher in 1999 at all three sites, the differences were not large, and the Simpson diversity index (1/D) was lower at Imagine in 1999 than in 1998. Stronger differences existed among sites, with Beatles consistently exceeding the other two sites in abundance, species richness and diversity of mycorrhizal fungi (Table 5.2). Imagine had lower estimates for species richness and lower diversity indices, while Green River was intermediate (Table 5.2).

5.3.3 EMF community composition

Among EMF taxa occurring frequently enough for statistical analysis, several EMF taxa occurred only at one of the three sites: *Tomentella viridula* (Green River), *Rhizopogon parksii* and *Tylospora fibrillosa* (Beatles) (Table 5.1). *Rhizopogon vinicolor*

I was absent in 1998 but was found at all three sites in 1999, a significant difference based on contingency table analysis of frequency using Fisher's Exact test (two-sided, P=0.017). *R. vinicolor* I was found only in the first half of the growing season from March through July (Fisher's two-sided exact test, P=0.010) as was *Piloderma byssinum* (Fisher's two-sided exact test, P=0.041). *Tuber* I was only found in the second half of the growing season from August through October (Fisher's two-sided exact test, P=0.027). Frequency of other taxa did not change significantly from year to year or from early to late season. When controlled for site and annual differences, soil moisture and pH were not significantly related to the frequency of the most abundant EMF taxa, with the exception of *Boletus zelleri*. Soil cores without *B. zelleri* were significantly drier than cores with *B. zelleri* (single-factor ANOVA, F=5.067, P=0.026).

Detrended correspondence analysis (DCA) partially segregated sites along the primary axis, with low values for Green River, intermediate values for Beatles, and high values for Imagine (Fig. 5.3). The primary axis had an eigenvalue of 0.618 and explained 44.3% of the variance in the species data. The secondary axis was only weakly related to the species data (eigenvalue=0.360, r^2=0.051).

5.4 Discussion

5.4.1 EMF taxa

The total of 33 EMF taxa observed in soil cores in this study is similar to the totals for other studies of roots of mature conifer trees subject to disturbance from harvesting, and well under the totals observed in undisturbed mature or old-growth Douglas-fir forests. Hagerman et al. (1999a) found 39 morphotypes in soil cores from forest edges in a subalpine forest in Interior British Columbia, while Stockdale (2000) found 41 morphotypes in thinned old-growth Douglas-fir forests in Oregon. Unthinned control stands of old-growth Douglas-fir had 61 morphotypes (Stockdale 2000), similar to the total of 69 morphotypes found in old-growth and mature Douglas-fir forests on Vancouver Island (Goodman and Trofymow 1998b). In contrast, Horton and Bruns

(1998a) found only 14 EMF taxa on Douglas-fir in mature mixed stands of Douglas-fir and bishop pine in California, possibly due to undersampling.

Two of the six most abundant EMF taxa in the present study, *C. geophilum* and *R. vinicolor*, were also among the three most abundant taxa observed in old-growth Douglas-fir stands by Goodman and Trofymow (1998b) and by Stockdale after thinning (2000). This was not surprising, as *C. geophilum* is a well-known generalist which occurs globally on a wide range of host species, and *R. vinicolor* is one of the most common associates of Douglas-fir trees (Zak 1971).

When the EMF communities of the residual trees were examined in spring of 2000, as described in Chapter 4, *Rhizopogon* spp. were not observed on excavated residual tree roots, while *Russula* species (particularly *R. nigricans*) were common. In soil cores, *Rhizopogon* species were uncommon at the two most recently harvested sites (Imagine and Green River), while at Beatles *Rhizopogon* species were abundant in soil cores, found on 32.7 ± 6.5% of root tips. *Rhizopogon* spp. were dominant on seedlings planted at all three sites, as described in Chapter 4. The higher abundance of *Rhizopogon* spp. at Beatles can probably be explained by an increased inclusion of roots from seedlings in soil cores compared to the other two sites. At Beatles, in the nine years since harvesting, planted Douglas-fir seedlings had developed extensive root systems.

5.4.2 EMF diversity and community structure

The average density of active EMF root tips in soil cores ($3.1 \pm 0.7\ l^{-1}$) in my study was orders of magnitude lower than in other studies in which densities were commonly in the hundreds of root tips per soil core (Visser 1995; Goodman and Trofymow 1998b; Jonsson et al. 1999a; Stockdale 2000). Nevertheless, similar densities were observed in broadcast-burned two-year-old clearcuts of Douglas-fir/western larch (*Larix occidentalis*) forests in Montana, where active EMF root tips declined precipitously with distance from the forest edge, from 15.8 tips per liter of soil 8 m inside the forest to 2.6 tips per liter 1.5 m into the clearcut to zero from 5 to 20 m into the clearcut (Harvey et al. 1980). While the soil cores in the present study were obtained over a comparable distance from a residual tree (2-6 m), the harvested areas were not broadcast burned. In small experimental gaps in lodgepole pine forests in Wyoming, active EMF root tip densities also declined to near zero, but only in the largest (30-tree) gaps (Parsons et al. 1994b).

The active EMF root tip density increased from 1998 to 1999 at all three sites. While this could reflect recovery from soil disturbance after harvesting, it seems unlikely since Douglas-fir seedlings planted < 6 m from the center trees maintained steady levels of EMF colonization during the same period, as described in Chapter 3. Roots from non-conifer plants were not quantified in soil cores, but many soil cores contained dense mats of grass, fern, or shrub roots, particularly from salal (*Gaultheria shallon*), which was common at Imagine and to some extent at Beatles. When portions of residual tree root systems were examined, feeder roots were surprisingly rare (see Chapter 4). Competition from understory plants proliferating in the increased light levels after harvesting could have displaced mature tree feeder roots to deeper soil horizons.

It is also possible that harvesting induces a period of reduced activity in residual trees, resulting in lower densities of feeder roots. The basal area increment of residual trees from old-growth Douglas-fir stands in Washington and Oregon was reduced by 15% compared to unharvested control stands for six years after harvesting (North et al. 1996). This was surprising since the release from competition from neighboring trees would be expected to increase growth rates. The above-ground and below-ground responses of residual trees to harvesting require further study.

5.4.4 EMF community composition

EMF community composition differed by site, as revealed by ordination via detrended correspondence analysis (DCA). Vectors showing the direction of change of EMF communities were not related in a consistent manner to the primary and secondary ordination axes. Each site followed its own unique trajectory over time. Temporal changes and soil variables were weakly correlated with the main axes (Fig. 5.3). The primary axis was positively correlated with seasonal changes from spring to fall ($r=0.403$, $r^2=0.16$) and negatively correlated with soil pH ($r= -0.377$, $r^2=0.14$). The secondary axis was negatively correlated with the percent soil moisture ($r= -0.538$, $r^2=0.29$) and positively correlated with soil pH ($r=0.431$, $r^2=0.19$).

When the placements of individual taxa were examined, species within the same genus were often separated. *Tomentella* spp. were arrayed along the secondary axis, with low values for *T. viridula*, intermediate values for *T. stuposa*, *T. sublilacina*, and *T. citrina*, and high values for *Tomentella* II (which occurred in only one soil sample) in the direction of decreasing soil moisture and increasing soil pH. *Russula* spp. were separated along the primary axis (as well as the secondary axis), with lower values for *R. bicolor* and higher values for *R. nigricans* and the other *Russula* taxa.

Community composition appeared to be more strongly influenced by larger-scale site differences than by soil microsite variation in moisture and pH, based on separation of samples by site in ordination. For soil pH this was not surprising, since the relatively small variation in soil pH from core to core may not have been biologically significant. My failure to find a strong relationship between soil moisture and EMF taxa was surprising, however. In ordination, soil moisture was correlated with the secondary axis, which was only weakly related to the species data. I expected that some EMF taxa would prove more resistant to drying and therefore remain active over a longer period. Instead, *Boletus zelleri* was the only taxon which appeared to be strongly influenced by soil moisture, predominating in moist soils. *B. zelleri* was also more frequent and abundant on Douglas-fir seedlings planted <6 m from residual trees and in microsites containing higher proportions of buried soil wood (see Chapter 4). Buried wood can act as a moisture reservoir (Amaranthus et al. 1989). This may explain the predominance of *B. zelleri* in wetter soil microsites. Trudell and Edmonds (2004) found in sporocarp surveys that *Boletus* was associated with higher moisture sites in Douglas-fir forests on the Olympic peninsula of Washington state.

Piloderma byssinum recurred each year in the spring while *Tuber* I recurred each year in the fall. Ordination reflected the same overall pattern. The shift in community composition from spring to fall was positively correlated with the primary axis, while the shift from 1998 to 1999 was negatively correlated with the same axis. Time since harvesting was not closely related to either of the main ordination axes. Changes in species composition over time may follow a seasonally cyclic pattern rather than a linear progression.

It is not surprising to find evidence of seasonal fluctuations in abundance of EMF taxa. Population studies suggest that EMF genets are subject to frequent turnover. Even in mature undisturbed forest, the majority of genets appear to be smaller than 3 m^2 (Redecker et al. 2001; Bergemann and Miller 2002; Kretzer et al. 2004). Rapid turnover of mycelium (Guidot et al. 2004) and mycorrhizal root tips (Zhou and Hogetsu 2002) has been observed under sporocarps of *Hebeloma cylindrosporum* and *Suillus grevillei*, respectively. The controls on this turnover have not been adequately explored, but could be related to seasonal changes in the soil environment or mediated by changes in the interaction of the host with the fungus (i.e., through sugar availability).

5.5 Conclusions

I did not assess mycorrhizas before harvesting, and therefore my study could not directly detect decreases in mycorrhizal abundance immediately after harvesting. Nevertheless, the low density of EMF root tips in soil cores near residual trees two to nine years after harvesting suggests that recovery from the impacts of harvesting may require many years. EMF abundance and diversity increased from year to year at each of the sites, but abundance and EMF species composition appeared to vary more by site than by years after harvesting. Abundance of EMF taxa was only weakly correlated with soil microsite characteristics of moisture and pH. Some EMF taxa were found only in early or late growing season, but the low density of EMF root tips made it difficult to detect other seasonal patterns. Further research is required to explore seasonal dynamics of EMF communities and the impacts of harvesting on the mycorrhizal status of residual trees.

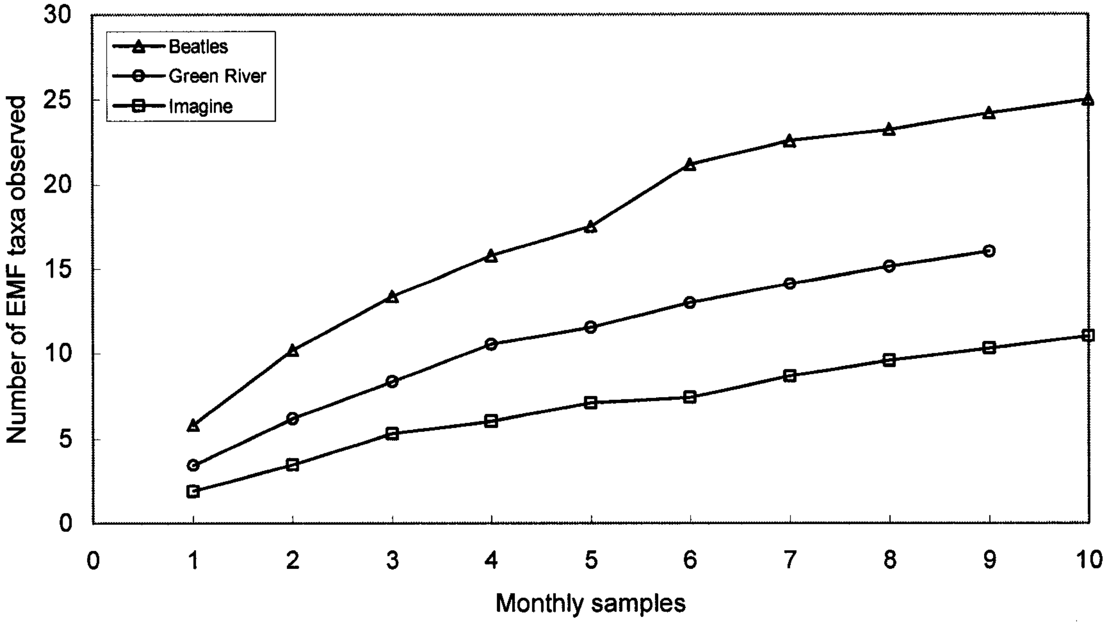

Fig. 5.1 'Species-area' curves for EMF taxa in soil cores.
Lines for each site show rate of encounter of new EMF taxa per month of soil sampling. Monthly samples are combined data from 4 replicates of 2 aggregated 10 cm diameter x 20 cm deep soil cores for a total volume of 13 l of soil per month at each site.

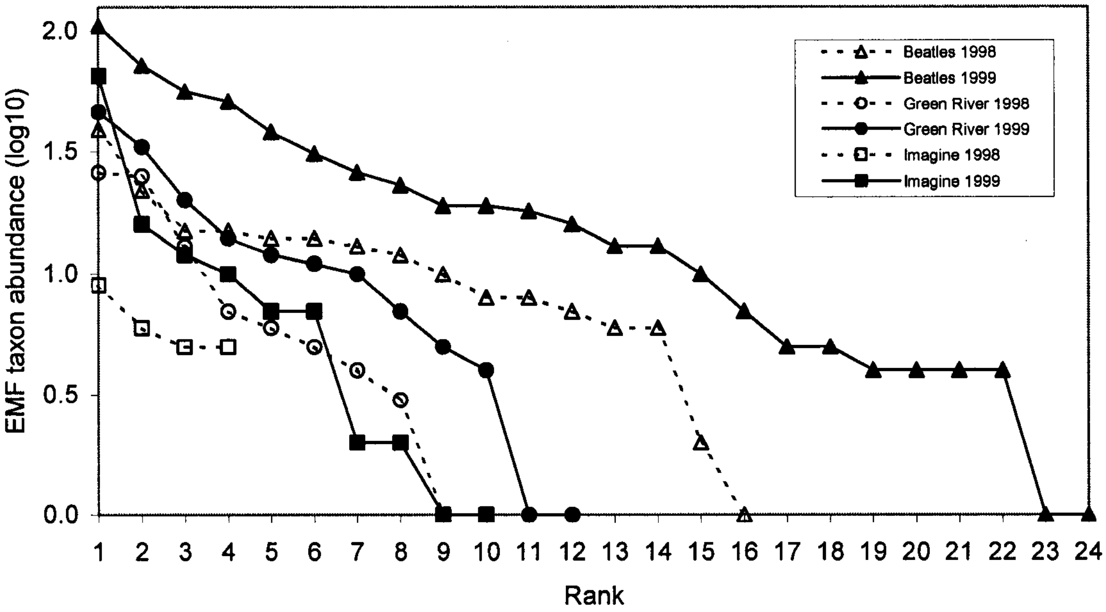

Fig. 5.2 Rank abundance of EMF taxa in soil cores near trees.
Slopes (B) of best fit line ($\log_{10}$(abundance) rank^{-1}): for Beatles, B=-0.07 in 1998 and B=-0.07 in 1999; for Imagine, B=-0.08 in 1998 and B=-0.19 in 1999; for Green River, B=-0.16 in 1998 and B=-0.14 in 1999.

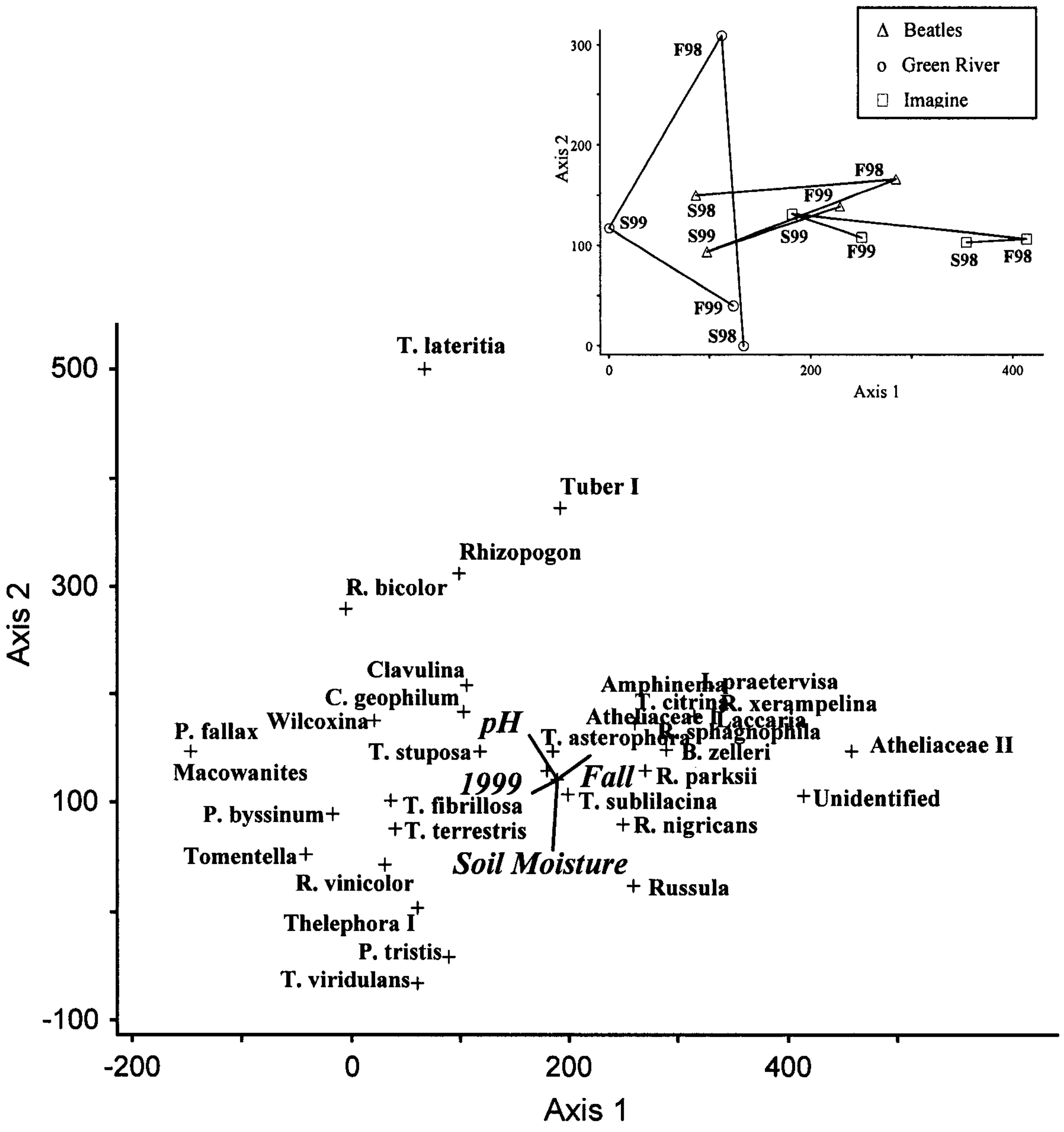

Fig. 5.3 Detrended correspondence analysis (DCA) of EMF taxa in soil cores near trees. Arcsin square-root transformed relative abundance of EMF root tips was analyzed. EMF taxa are plotted with joint plot lines representing r^2 values for the relationship between both axes and environmental variables with r^2>10%: soil moisture and pH, annual change from 1998 to 1999, and seasonal change from spring to fall. Inset: seasonal samples are plotted: S98=spring 1998, F98=fall 1998, S99=spring 1999, F99= fall 1999. Vectors represent change over time at each site.

Chapter 6. Summary and Conclusions

The purpose of this study was to evaluate the effectiveness of partial canopy retention (residual forestry) in maintaining biodiversity of EMF communities in managed Douglas-fir forests in western Washington. EMF communities of residual trees were assessed directly at three sites in the Cedar and Green River watersheds, by partially excavating tree root systems. In addition, the effects of proximity to residual trees on Douglas-fir seedling growth, mycorrhizal colonization levels, and diversity of the EMF community on seedlings were evaluated over several growing seasons. Finally, soil cores traced post-harvesting changes in EMF communities of residual forests.

Growth of seedlings was affected in a variety of ways by proximity to residual trees. Root growth rates were highest for seedlings <6 m from trees, especially in the first year after planting. These seedlings devoted more resources to roots than to shoots, developing higher root to shoot biomass ratios than seedlings >16 m from trees. On average, stem growth was lower for seedlings <6 m from trees, with significant differences in height, diameter, and stem volume developing by the third year after planting. Site differences strongly influenced the effect of proximity to trees, however. For the site with the lowest density of understory vegetation, <6 m seedlings had higher stem growth rates than >16 m seedlings. Seedlings <6 m from trees sustained a greater proportion of active mycorrhizal roots, which was correlated with increased root and stem growth in the first year after planting, but with decreased stem growth in the second year.

Both <6 m and >16 m seedlings rapidly acquired a diverse component of EMF taxa after planting, although nursery taxa persisted as well. Seedlings <6 m from residual trees had a total of 47 EMF taxa and averaged 4.1 taxa per seedling, while <16 m seedlings had 38 total taxa and averaged only 3.3 taxa per seedling. *Rhizopogon* spp. were dominant on both <6 m and >16 m seedlings, but were less abundant on <6 m seedlings. Instead, <6 m seedlings were more likely to share EMF taxa with residual trees. EMF taxa of residual trees, including *Russula* spp. (particularly *R. nigricans*), *Tylospora* spp., *Tomentella* spp., and *Boletus zelleri*, were more frequent on <6 m seedlings than on >16 m seedlings. In contrast, the EMF community of >16 m seedlings was more similar to the less diverse EMF community of greenhouse seedlings.

Seedlings <6 m from residual trees had higher mycorrhizal colonization levels, which may have partially accounted for the higher diversity of the EMF community. Nevertheless, proximity to residual trees appeared to directly enhance mycorrhizal diversity of seedlings by increasing the evenness of the EMF community structure.

I did not measure mycorrhizal colonization of residual trees before harvesting, therefore I cannot comment on changes in mycorrhizal abundance immediately after harvesting. Nevertheless, the low density of EMF root tips in soil cores <6 m from residual trees two to nine years after harvesting suggested that recovery from the effects of harvesting may require many years. EMF abundance and diversity increased from year to year at each of the sites, but abundance and EMF species composition appeared to vary more by site than by time elapsed post-harvesting. Some EMF taxa were found only early or late in the growing season, but the low density of EMF root tips made it difficult to detect other seasonal patterns. Further research is required to distinguish between seasonal changes of EMF communities and post-harvesting successional changes.

Residual trees fulfill multiple purposes in recently harvested forests. First, they retain a unique EMF community on their root systems. Second, they influence the species composition and enhance the diversity of EMF communities of adjacent seedlings. This could have important functional consequences not just for host plants but for the ecosystem as a whole as harvested forests regenerate. Forests with more diverse mycorrhizal communities might respond differently to future disturbance or climate change.

The long-term benefits of residual forestry for Douglas-fir regeneration require further investigation. Nevertheless, it appears likely that residual trees would have lasting impacts on mycorrhizal diversity as stands recover from the effects of harvesting. The higher mycorrhizal diversity of seedlings near residual trees supports the effectiveness of residual forestry as a management tool in conserving biodiversity.

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Appendix A. Morphological Descriptions of EMF Taxa on Roots of Douglas-fir Seedlings and Trees.

EMF Taxon	Root tip branching pattern
Amanita I	monopodial pinnate, tortuous
Amphinema byssoides	monopodial pinnate with widely spaced branches, straight to slightly tortuous
Amphinema I	simple, straight
Ascomycota I	various
Atheliaceae I	simple, monopodial pinnate or pyramidal, straight to slightly bent
Atheliaceae II	various
Boletus zelleri	irregular to coralloid, tortuous, large clusters
Cenococcum geophilum	simple or singly-branched; rarely monopodial pinnate or pyramidal
Clavulina I	monopodial pinnate to iirregular or coralloid, large clusters, widely spaced branches, straight or bent
Cortinarius cinnamomeus	simple to coralloid tortuous
Hebeloma album gp.	commonly simple, sometimes monopodial pinnate, straight to tortuous
Hymenoscyphus ericae	simple, beaded or tapered at tip
Inocybe I	simple, slightly tortuous
Inocybe II	irregular tortuous
Inocybe praetervisa gp.	irregular tortuous
Inocybe sierraensis gp.	irregular tortuous, forming large clusters with widely spaced branches
Inocybe sindonia gp.	various
Laccaria I	simple to monopodial pinnate, slightly tortuous
Lactarius I	monopodial pinnate, bent to tortuous, large clusters
Macowanites americanus	simple to coralloid, bent, large clusters
Melanogaster I	irregular tortuous
Peziza I	simple beaded, tapered tip
Piloderma byssinum	simple or monopodial pinnate, straight
Piloderma fallax	simple, uncommonly monopodial pinnate, bent
Pseudotomentella nigra	single-branched or monopodial pinnate, bent
Pseudotomentella tristis	simple to monopodial pinnate, straight
Rhizopogon I	monopodial pinnate to irregular, bent to tortuous
*Rhizopogon parksii**	coralloid or tuberculate
Rhizopogon spp.	simple to monopodial pinnate with short, tightly packed branches, to tuberculate
Rhizopogon villosulus	various
Rhizopogon vinicolor I	tuberculate
Russula adusta	irregular slightly tortuous
Russula amoenipes gp.	monopodial pinnate with widely spaced branches, straight at right angles
Russula bicolor	monopodial pinnate to irregular, closely-spaced branches, slightly tortuous
Russula chloroides	coralloid tortuous
Russula nigricans	monopodial pinnate to coralloid, tortuous, large clusters >1cm axis
Russula sphagnophila gp.	monopodial pinnate to coralloid, tortuous, large clusters >1cm axis
Russula spp.	monopodial pinnate or irregular, slightly curved to tortuous
Sebacina I	coralloid tortuous, large clusters to 1 cm
Thelephora I	simple to coralloid, large clusters to 2 cm
Thelephora terrestris	simple to irregular, commonly straight or slightly bent, with widely spaced branches
Tomentella ellisii gp.	simple, monopodial pinnate, or irregular; straight to slightly bent
Tomentella II	irregular to coralloid, tortuous
Tomentella spp.	simple to irregular, bent to tortuous
Tomentella stuposa	monopodial pinnate, bent to tortuous, large clusters to 1 cm axis
Tomentella sublilacina	monopodial pinnate, slightly bent to tortuous, widely spaced branches, large clusters axis >1cm
Tomentella viridula	monopodial pinnate to irregular
Tomentellopsis I	straight
Truncocolumella citrina	monopodial pinnate straight to bent, tightly branching
Tuber I	monopodial pinnate to irregular, tortuous
Tylospora asterophora	monopodial pinnate to irregular, sometimes bent or tortuous, tapering at the tip
Tylospora fibrillosa gp.	monopodial pinnate straight to slightly tortuous
Wilcoxina spp.	simple, beaded or tapered at tip

*could not be distinguished from *Rhizopogon vinicolor* var. II

EMF Taxon	Mantle color
Amanita I	brilliant orange-yellow
Amphinema byssoides	orange-yellow to strong yellow brown
Amphinema I	orange-yellow
Ascomycota I	various
Atheliaceae I	vivid or brilliant orange-yellow to brown
Atheliaceae II	various
Boletus zelleri	vivid yellow to brilliant orange-yellow, some with red tones or rarely with green tones
Cenococcum geophilum	black
Clavulina I	usually creamy white with yellow and pink tone, sometimes light to medium orange
Cortinarius cinnamomeus	orange-yellow
Hebeloma album gp.	dark or deep orange-yellow to brilliant orange-yellow
Hymenoscyphus ericae	various
Inocybe I	pink-yellow
Inocybe II	light, vivid, or deep orange-yellow
Inocybe praetervisa gp.	pink-yellow to medium orange-yellow with pink tones
Inocybe sierraensis gp.	light yellow to orange-yellow
Inocybe sindonia gp.	yellowish pink
Laccaria I	commonly brilliant orange-yellow, sometimes medium orange or vivid yellow
Lactarius I	brown or deep orange, sometimes strong to brilliant orange-yellow
Macowanites americanus	medium orange, or strong to brilliant orange-yellow
Melanogaster I	yellow gold bruising to dark orange-yellow; or deep orange to red-brown
Peziza I	various
Piloderma byssinum	yellow
Piloderma fallax	yellow
Pseudotomentella nigra	medium yellow-brown to olive brown, dark or deep orange-yellow
Pseudotomentella tristis	black to dark brown
Rhizopogon I	black patchy with light orange or light yellow; white reflective patches, sometimes partially covered by pinkish felt
*Rhizopogon parksii**	(reddish) black with white reflective patches under silver brown felt
Rhizopogon spp.	black with white reflective patches bruising to yellow and gray to brown felty covering
Rhizopogon villosulus	various
Rhizopogon vinicolor I	dark red brown to black with reflective white patches, under dark felt
Russula adusta	orange-yellow
Russula amoenipes gp.	light to strong orange-yellow
Russula bicolor	light, medium, or brown orange base, light yellow tips
Russula chloroides	orange-yellow
Russula nigricans	commonly medium or brilliant orange-yellow; sometimes dark or deep orange-yellow or medium orange
Russula sphagnophila gp.	commonly light or brilliant orange-yellow; sometimes dark, deep, or medium orange yellow or with pink tones
Russula spp.	commonly strong to deep orange-yellow; sometimes light to pale orange or brilliant orange-yellow
Sebacina I	brillliant orange-yellow to strong orange
Thelephora I	creamy white with yellow and pink tones to orange-yellow
Thelephora terrestris	commonly light to brilliant orange-yellow; sometimes strong or deep orange-yellow or brown to medium orange
Tomentella ellisii gp.	commonly light orange-yellow; sometimes strong yellow-brown to strong orange, often ligther yellow at tips
Tomentella II	commonly deep to strong orange yellow; sometimes brown or orange
Tomentella spp.	commonly orange-yellow with pink tones; sometimes orange to brown
Tomentella stuposa	dark red brown to black with red tones
Tomentella sublilacina	medium orange to brilliant orange yellow
Tomentella viridula	commonly deep orange with vivid orange-yellow tips; sometimes strong yellow-brown to strong brown
Tomentellopsis I	orange-yellow
Truncocolumella citrina	yellow gold some with green tones
Tuber I	commonly deep orange or deep orange-yellow; sometimes brilliant orange-yellow, strong orange or brown-orange
Tylospora asterophora	strong orange or strong yellow-brown or vivid orange-yellow
Tylospora fibrillosa gp.	orange-yellow to orange, sometimes yellow at tip
Wilcoxina spp.	brown with creamy yellow tips

* could not be distinguished from *Rhizopogon vinicolor* var. II

EMF Taxon	Mantle texture	Mantle luster
Amanita I	felty	reflective white, in patches
Amphinema byssoides	cottony	reflective white, well developed
Amphinema I	cottony	
Ascomycota I		
Atheliaceae I	cottony; rarely, smooth	reflective white, sometimes patchy
Atheliaceae II		
Boletus zelleri	smooth	reflective gold or bronze
Cenococcum geophilum	warty, grainy	shiny
Clavulina I	generally smooth, sometimes felty or cottony	shiny
Cortinarius cinnamomeus	cottony	reflective, white or yellow, in patches
Hebeloma album gp.	cottony	matte or reflective white
Hymenoscyphus ericae		
Inocybe I	smooth	some reflective white patches
Inocybe II	smooth	shiny
Inocybe praetervisa gp.	smooth	shiny with rare reflective patches
Inocybe sierraensis gp.	felty	matte to shiny
Inocybe sindonia gp.	smooth or velvet	
Laccaria I	smooth to velvet or felty	reflective white patches
Lactarius I	smooth	matte with reflective white patches
Macowanites americanus	smooth or felty	
Melanogaster I	felty	reflective gold
Peziza I		
Piloderma byssinum	smooth or cottony	reflective white
Piloderma fallax	cottony	reflective white
Pseudotomentella nigra	smooth, felty, or woolly	shiny
Pseudotomentella tristis	felty or grainy	matte to shiny
Rhizopogon I	warty, felty, sometimes cottony	matte below reflective white patches
*Rhizopogon parksii**	warty, felty	reflective white patches
Rhizopogon spp.	warty, felty	reflective white
Rhizopogon villosulus		
Rhizopogon vinicolor I	felty	matte with reflective white patches
Russula adusta	smooth or fine velvet	rare white reflective patches
Russula amoenipes gp.	felty to cottony	
Russula bicolor	smooth, finely grainy, or short velvet	shiny/translucent
Russula chloroides	smooth	reflective white patches
Russula nigricans	felty or velvet	some reflective white patches
Russula sphagnophila gp.	smooth to finely grainy or velvet	shiny, some white reflective patches
Russula spp.	thick felty, velvet, or smooth	shiny, some white reflective patches
Sebacina I	velvet, cottony	reflective white patches
Thelephora I	smooth or felty	rare reflective white patches
Thelephora terrestris	smooth, felty, or velvet	shiny with some reflective patches
Tomentella ellisii gp.	felty or cottony	shiny, or some with white reflective patches
Tomentella II	long velvet	matte or shiny
Tomentella spp.	cottony or velvet	shiny with some white reflective patches
Tomentella stuposa	grainy	shiny
Tomentella sublilacina	felty or (long) velvet	shiny, rare reflective white patches
Tomentella viridula	velvet or grainy	shiny
Tomentellopsis I	felty	reflective white patches
Truncocolumella citrina	felty	reflective yellow or green-yellow
Tuber I	smooth or velvet	shiny
Tylospora asterophora	smooth	shiny or reflective white
Tylospora fibrillosa gp.	smooth	shiny
Wilcoxina spp.	webbed or cottony network; no mantle	shiny

* could not be distinguished from *Rhizopogon vinicolor* var. II

EMF Taxon	Description of emanating hyphas (EMH)	EMH type**
Amanita I	long curled cystidia	short
Amphinema byssoides	white to yellow, thick cottony mycelium, extending from tip 2-3 X tip diameter; large white to yellow rhizomorphs	medium fringe
Amphinema I	white cottony mycelium extending from tip 2-3 X tip diameter	short
Ascomycota I	white tufted mycelium;thin, poorly developed mantle	short
Atheliaceae I	thick cottony mycelium extending to 3x tip diameter; white reflective rhizomorphs	medium fringe
Atheliaceae II		short
Boletus zelleri	large yellow to brown rhizomorphs, usually tight, branching at right or acute angles	long
Cenococcum geophilum	stiff wiry black hyphal cords or bristles	short
Clavulina I	short black hyphal cords along mantle surface; sometimes white cottony mycelium extending up to 1X tip diameter	short
Cortinarius cinnamomeus	abundant white or yellow rhizomorphs	medium fringe
Hebeloma album gp.	copious cottony mycelium, white to pale or gray yellow to light orange-yellow, extending up to 2 mm from tip; rare white reflective, loose rhizomorphs	short
Hymenoscyphus ericae		short
Inocybe I	rare single emanating hyphae	contact
Inocybe II	no rhizomorphs or emanating hyphae	contact
Inocybe praetervisa gp.		contact
Inocybe sierraensis gp.	none	short
Inocybe sindonia gp.	yellow mycelium	short
Laccaria I	rare yellow to orange loose rhizomorphs, otherwise no emh	medium fringe
Lactarius I	none	contact
Macowanites americanus	some wiry black hyphal cords along mantle	contact
Melanogaster I	tight yellowgold or brown rhizomorphs with right angled branches every 2 mm	long
Peziza I		short
Piloderma byssinum	abundant cottony white hyphal fans to 2 cm, loose white reflective rhizomorphs	medium fringe
Piloderma fallax	abundant thick cottony white or yellow mycelium, yellow or white reflective rhizomorphs	medium fringe
Pseudotomentella nigra	abundant thick woolly dark gray mycelium up to 3 mm, emanating from base of tip	short
Pseudotomentella tristis	thick woolly brown mycelium, deep to strong brown rhizomorphs	medium fringe
Rhizopogon I	abundant, long brown-black hyphal bundles--restricted point	long
*Rhizopogon parksii**	yellow or black loose rhizomorphs bifurcating every 1-2 cm	long
Rhizopogon spp.	gray, yellow, or brown rhizomorphs branching at acute angles	long
Rhizopogon villosulus		long
Rhizopogon vinicolor I	rhizomorphs, commonly black, but sometimes white, gray, yellow or brown	long
Russula adusta	none	contact
Russula amoenipes gp.	some with thick cottony mycelium	contact
Russula bicolor	cystidia, some tufts of cottony white mycelium, rare black hyphal cords	short
Russula chloroides		contact
Russula nigricans	some with dense short cystidia, thick black hyphal cords on mantle, occasionally with yellow rhizomorphs	medium smooth
Russula sphagnophila gp.	sometimes cystidial; wiry black hyphal cords on mantle	contact
Russula spp.	none; or with black hyphal cords, cottony mycelium, or yellow rhizomorphs	medium fringe
Sebacina I	some with abundant white cottony mycelium, rare white reflective rhizomorphs	medium fringe
Thelephora I	sparse white cottony mycelium	short
Thelephora terrestris	none, or with sparse cottony white mycelium, or with olive-brown to yellow to white loosely-woven rhizomorphs with acute branching angles	medium fringe
Tomentella ellisii gp.	none, or with sparse cottony white mycelium, or with short cystidia	short
Tomentella II	long cystidia, some with white to brown-gray cottony mycelium; rarely, with black hyphal cords	short
Tomentella spp.	woolly brown mycelium and wiry brown hyphal cords with brown rhizomorphs; or, cottony white mycelium	medium fringe
Tomentella stuposa	rare brown mycelium; tight red-brown to brown rhizomorphs branching at acute angles	medium smooth
Tomentella sublilacina	long cystidia	short
Tomentella viridula	short straight dense cystidia, or longer curled cystidia; uncommon wisps of white mycelium	short
Tomentellopsis I	none	contact
Truncocolumella citrina	large black, gray-brown, or olive-green rhizomorphs	long
Tuber I	some with cystidia, others with patchy white or buff felty mycelium extending to 3 mm	short
Tylospora asterophora	some with cottony white mycelium	short
Tylospora fibrillosa gp.	none, or rarely with single fine white to yellow hyphae	contact
Wilcoxina spp.	some thick white or yellow cottony mycelium	short

* could not be distinguished from *Rhizopogon vinicolor* var. II

Appendix B. Phylogenetic Placement of Ribosomal Sequences from EMF Taxa of Douglas-fir Trees and Seedlings.

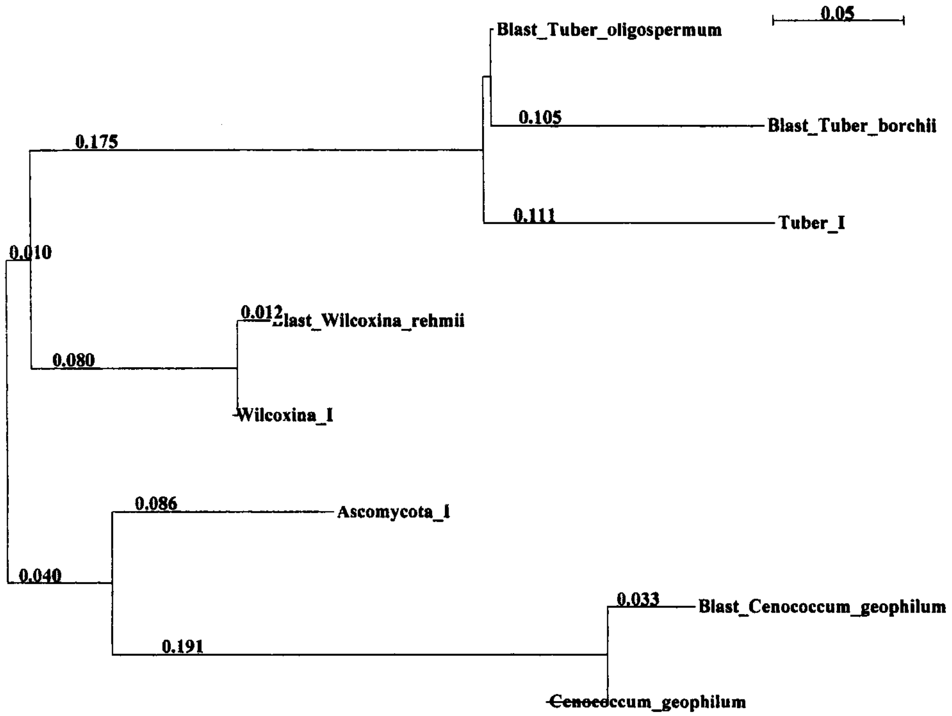

Fig. B.1 Neighbor-joining tree of internal transcribed spacer (ITS) ribosomal RNA (rRNA) sequences from EMF taxa. Sequences included a portion of the 18S rRNA gene, the entire ITS-I, 5.8S rRNA, and ITS-II region, and a portion of the 28S rRNA gene. Taxon names preceded by blast were obtained via BLAST searches from the www.ncbi.nih.gov database. Taxon names preceded by FB are from sporocarps collected at the field sites. Putative taxon names are shown for Douglas-fir root tips collected at the field sites. Branch lengths (substitutions per basepair) are shown for each branch.

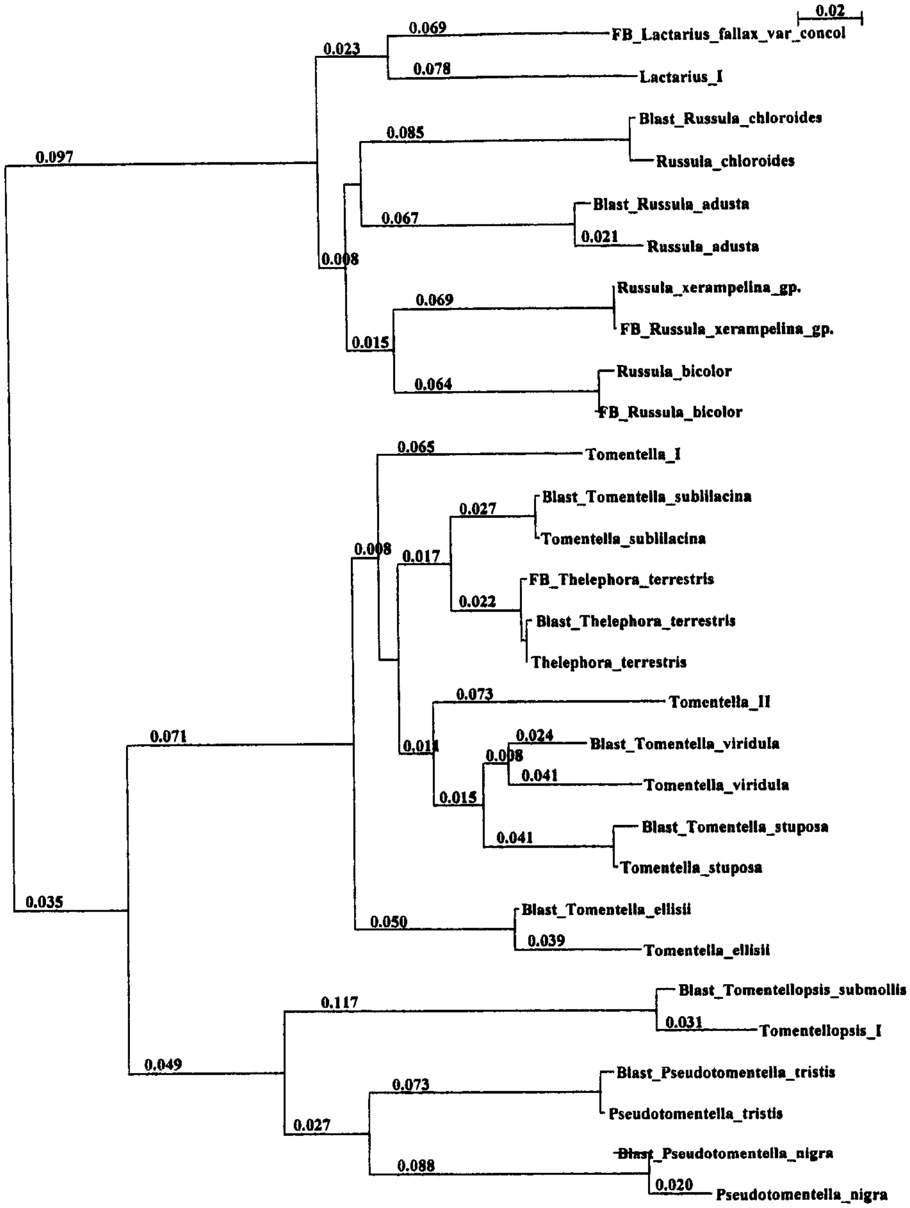

Fig. B.1 (continued)

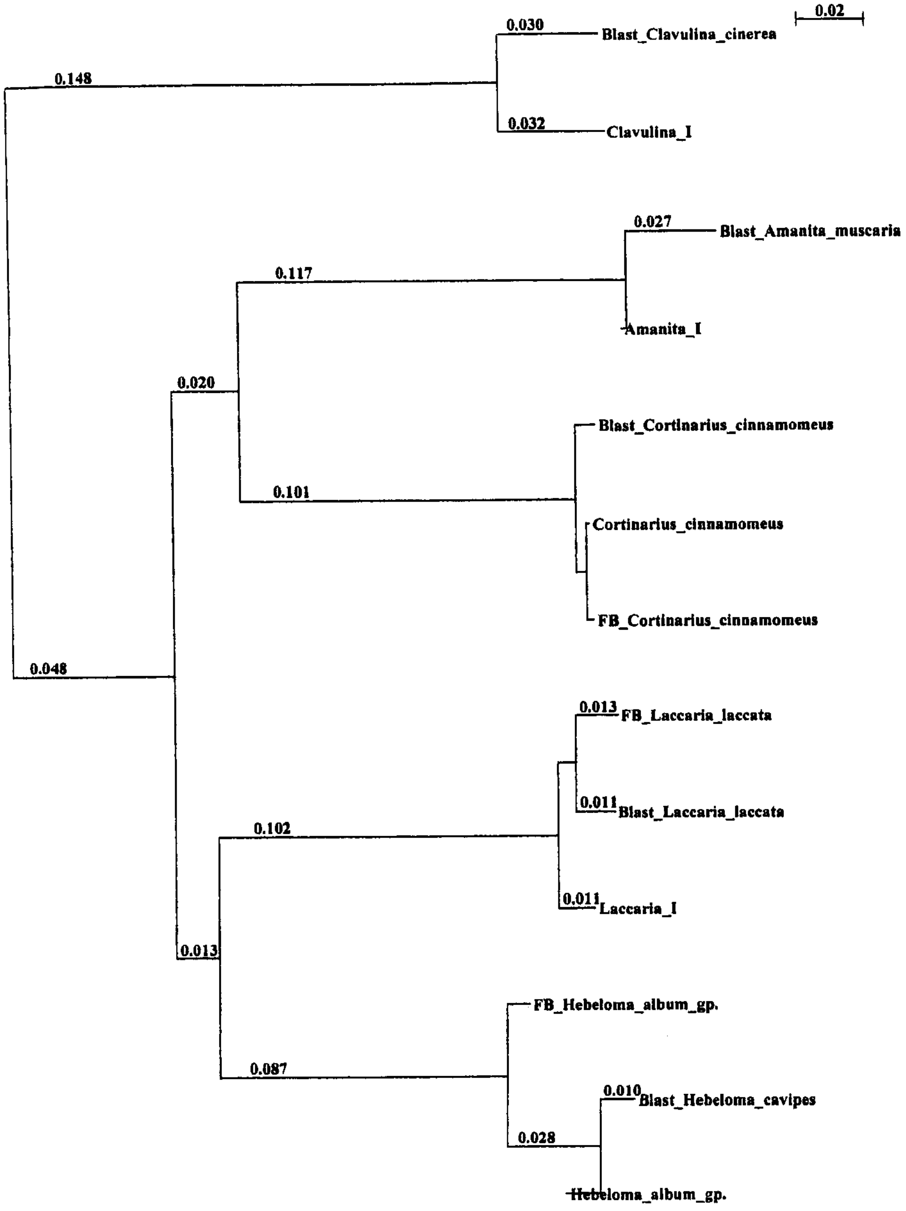

Fig. B.1 (continued)

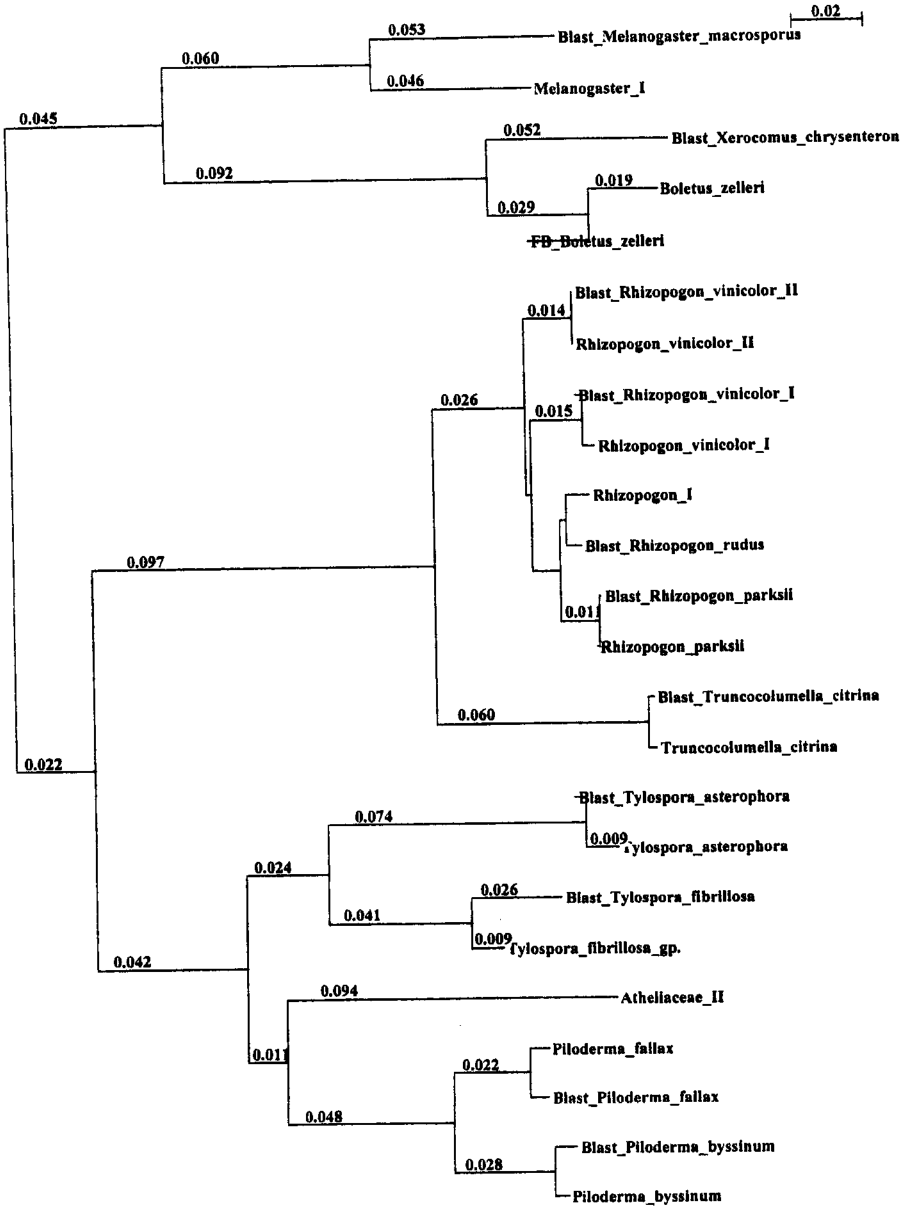

Fig. B.1 (continued)

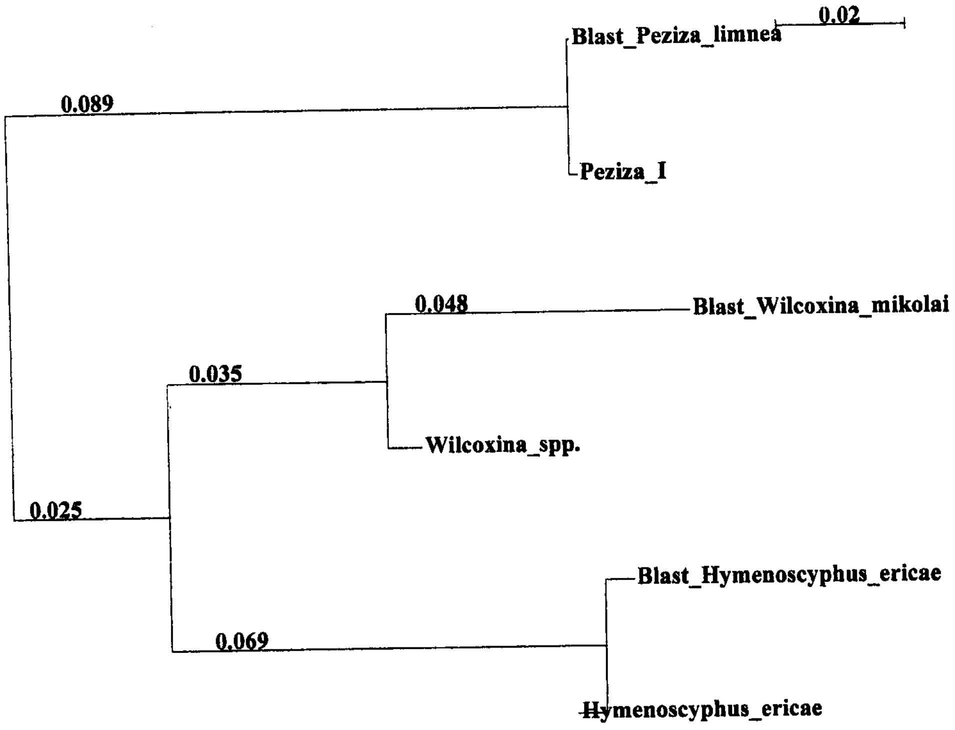

Fig. B.2 Neighbor-joining tree of large subunit (28S) ribosomal RNA (rRNA) sequences from EMF taxa. Sequences included a portion of the large subunit (28S) rRNA gene. Taxon names preceded by blast were obtained via BLAST searches from the www.ncbi.nih.gov database. Taxon names preceded by FB are from sporocarps collected at the field sites. Putative taxon names are shown for Douglas-fir root tips collected at the field sites. Branch lengths (substitutions per basepair) are shown for each branch.

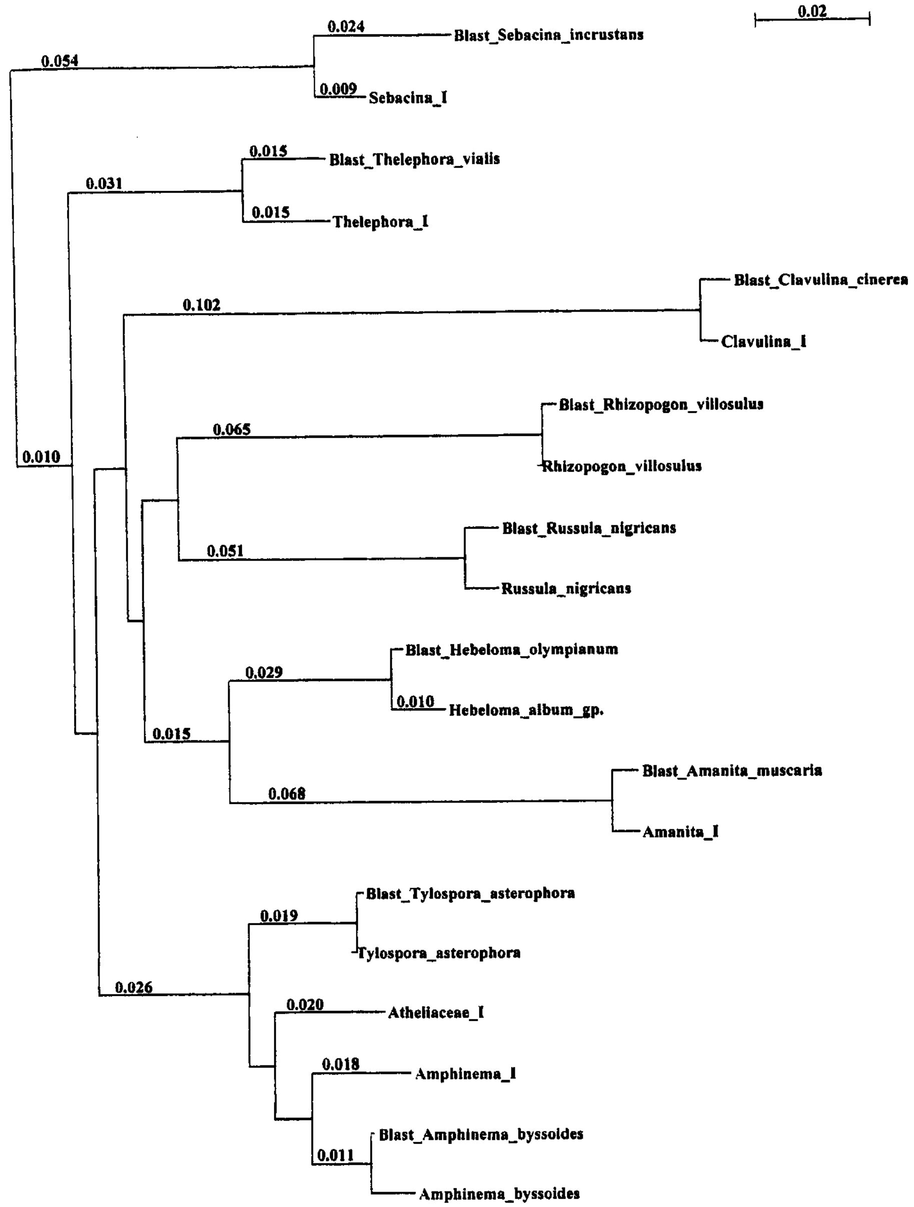

Fig. B.2 (continued)

Appendix C. GenBank Accession Numbers of Ribosomal Sequences from Sporocarps and Mycorrhizal Root Tips Collected at the Three Study Sites.

Accession Number	Specimen Voucher #	Taxonomic Classification*	rRNA type	Sporocarp or Mycorrhiza
AY356323	EC172-C1	*Cortinarius subolivascens*	ITS	Sporocarp
AY750156	EC174-C19	*Laccaria proxima*	ITS	Sporocarp
AY750157	EC175-C25	*Inocybe lacera*	ITS	Sporocarp
AY750158	EC176-C41	*Boletus zelleri*	ITS	Sporocarp
AY750159	EC177-C56	*Cortinarius cinnamomeus*	ITS	Sporocarp
AY750160	EC178-C59	*Lactarius fallax var. concolor*	ITS	Sporocarp
AY750161	EC179-C83	*Russula bicolor*	ITS	Sporocarp
AY750162	EC180-C97	*Hebeloma album gp.*	ITS	Sporocarp
AY750163	EC181-C128	*Thelephora terrestris*	ITS	Sporocarp
AY750164	EC182-C130	*Russula xerampelina gp.*	ITS	Sporocarp
AY750165	EC252-C42	*Amanita constricta gp.*	ITS	Sporocarp
AY750166	EC253-C44	*Tricholoma atroviolaceum*	ITS	Sporocarp
AY750167	EC183-C144	*Macowanites iodiolans* gp.	ITS	Sporocarp
AY750168	EC333-C64	*Inocybe* sp.	LSU	Sporocarp
AY750169	EC211-B637	*Inocybe* sp.	ITS	Mycorrhiza
AY751555	EC189-B138	*Inocybe* sp.	ITS	Mycorrhiza
AY751556	EC258-B207	*Inocybe* sp.	ITS	Mycorrhiza
AY751557	EC99-A59	*Inocybe* sp.	ITS	Mycorrhiza
AY751558	EC279-A565	*Inocybe* sp.	ITS	Mycorrhiza
AY751559	EC261-B293	*Tuber* sp.	ITS	Mycorrhiza
AY751560	EC267-B452	*Lactarius* sp.	ITS	Mycorrhiza
AY751561	EC200-B381	*Tomentella* sp.	ITS	Mycorrhiza
AY751562	EC117-A52	*Tomentella* sp.	ITS	Mycorrhiza
AY751563	EC185-B92	*Clavulina* sp.	ITS	Mycorrhiza
AY751564	EC243-B603	Atheliaceaea	ITS	Mycorrhiza
AY751565	EC299-B138	*Inocybe* sp.	LSU	Mycorrhiza
AY751566	EC325-B345	*Inocybe* sp.	LSU	Mycorrhiza
AY751567	EC364-B318	Atheliaceae sp.	LSU	Mycorrhiza
AY751568	EC361-B263	*Amphinema* sp.	LSU	Mycorrhiza

*For mycorrhizal root tips, based on closest neighbor from a BLAST search.

VITA

Erica Cline was born in Seattle, Washington and spent many happy days hiking in the rugged mountains and towering forests there. She is currently residing in Maryland, where she is learning to cherish the gentler hills and deciduous forests of the eastern seaboard. She lived in the Netherlands for three years as a child. After earning a Bachelor of Science in Biology at the University of Puget Sound in Tacoma, Washington, she returned to the Netherlands to receive a Master of Science in Cell Biology from the Rijks Universiteit Leiden. In 2004 she earned a Doctor of Philosophy from the College of Forest Resources at the University of Washington in Seattle.