Ectomycorrhizal Communities of Douglas-fir and Paper Birch Along a Gradient of Stand Age Following Clearcutting and Wildfire in the Interior Cedar-Hemlock Zone, Southern British Columbia

by

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Abstract

Ectomycorrhizal (ECM) communities of Douglas-fir and paper birch were characterized in 5-, 26-, 65-, and 100-year-old stands using ECM root tip morphology and ITS region DNA sequences. Stands disturbed by wildfire (all age classes) and clearcutting (two youngest age classes) were studied (4 replicates per stand type) in the Interior Cedar-Hemlock forests of southern British Columbia. ECM community species richness on Douglas-fir differed with stand age, being three times higher in 65- and 100-yr-old stands than 5-yr-old stands; 26-yr-old stands were intermediate. In the 5-yr-old stands, the ECM community of paper birch had ~70% higher species richness than Douglas-fir. Roots of sprouting paper birch stumps may support mycorrhizae or inocula that persist through disturbance. Overall ECM diversity increased substantially from 5- to 26-yr-old stands, but changed little with further stand age increase. ECM community composition and structure shifted from 5- to 26-yr-old stands and continued to change from 26- to 65-yr-old stands, including increases in *Russula* and *Piloderma* relative abundances. Similar ECM communities occurred on 65- and 100-yr-old stands. *Cortinarius* and *Hebeloma* were nearly absent in 5-yr-old stands and peaked in relative abundance in 26-yr-old stands. Generally, patterns in relative abundance of fungal taxa with stand age paralleled those in frequency.

Host-specific ECM fungi were most dominant in the youngest stands, particularly *Rhizopogon vinicolor*-type on Douglas-fir and *Lactarius pubescens* on paper birch. Host-generalists were more abundant on paper birch than Douglas-fir at younger ages, suggesting that paper birch may be important in the establishment of these fungi on Douglas-fir. Paper birch also had an important positive influence on ECM fungal diversity in 5-yr-old stands, which should be considered in forest management. There was no difference in ECM diversity between clearcut and wildfire origin sites, although community structure differed slightly between these disturbances in 5-yr-old stands. Within the range of ages studied, it appears that the ECM fungal community changed little after 65 years following disturbance. Available P was correlated with abundance of two dominant taxa, but available N and P, mineralizable N, and organic P were not related to ECM diversity or community structure.

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Dedication

I wish to dedicate this work to my family, who are just as supportive and loving as one could ever hope a family to be, and to Dr. David Largent, who sparked my earliest interest in fungi and taught me an awful lot about them.

Chapter 1 Introduction and Literature Review

Introduction

Ectomycorrhizal (ECM) fungi are an extremely diverse group of organisms comprised mainly of species in the Basidiomycota, with a smaller number of species in the Ascomycota. These fungi exhibit a wide variety of above-ground reproductive structures, including mushrooms, cups (apothecia), polypores, and resupinate forms (appressed to the surface) on dead wood. Most fungi with hypogeous (below-ground) fruiting bodies, known as truffles or false truffles, are also ectomycorrhizal. It has been estimated that over 5000 fungal species are in the ECM group (Molina *et al.*, 1992), and the communities of ECM fungi are often several times more diverse than associated communities of vascular plants. The vast majority of fine roots of all conifer species in the Pinaceae, as well as some broadleaf genera such as *Betula* , *Quercus*, and *Fagus*, form a symbiotic[1] relationship with these fungi. Other broadleaf hosts, such as those in the Salicaceae, form symbioses with both ECM and arbuscular mycorrhizal (AM) fungi. Most ECM fungi form an obvious mantle (sheath of tissue) around the terminal and penultimate root segments, thus interfacing between fine roots and soil. They also form a hartig net, a collective of ramified fungal cells in the intercellular space of the root epidermis and cortex, which is always present even though a mantle is sometimes not.

The term "mycorrhiza" has often been applied only to mutualistic symbioses (Allen, 1991). However, some mycorrhizal fungi, including ECM fungi, may act as parasites, mutualists, or somewhere in between, dependent upon the context (e.g. Jonsson *et al.*, 2001; Hashimoto & Hyakumachi, 2001; Johnson *et al.*, 1997). Jones & Smith (2004), in their thorough review of this topic, concluded that "mycorrhizas should be defined on a structural or developmental basis and any requirement to demonstrate mutualism must be eliminated". A central problem in the field of mycorrhizal research, and one that led Jones & Smith (2004) to this conclusion, is that ECM effects on plant host fitness cannot be studied under natural conditions in the field. Here, I use the structural definition for the term "ectomycorrhizal"; i.e. an ECM root is one that has a hartig net, fungal mantle, or both.

[1] The term "symbiosis" is used here to mean two organisms living in intimate physical contact; there is no implication of mutualism in this term.

Science is continually discovering new below-ground ECM relationships between fungi and plants. It provides insight into the range of ECM fungal habitats and a fundamental reference for more detailed studies of their specific functions in ecosystems. Although some ECM fungi can grow in culture without a host plant association, it is widely accepted that most are obligate symbionts in nature and require connections to hosts in order to grow vegetatively (Fleming, 1984) and sexually reproduce (Lamhamedi *et al.*, 1994). Many fungi can associate with a variety of host plants, but a substantial proportion of them are also restricted to a particular host species or genus. Thus, the ecological niches for many species of ECM fungi are largely defined by the range of their hosts. Maintenance of tree diversity is therefore essential for maintaining ECM fungal diversity (Massicotte *et al.*, 1999).

Mycorrhizal research dates back to the late 1800's, and great advances have been made in the field since then. Describing effects of ECM fungi on host plants has depended on isolating the fungi in pure culture and using them to inoculate hosts in controlled experiments. Researchers have inoculated host trees with a variety of ECM fungi to examine their abilities to take up nutrients from natural substrates and model compounds, as well as to transport nutrients and carbon to and between different host trees. Such studies have been carried out mostly in artificial conditions. Meanwhile, mycologists and ecologists have described host species and habitats these fungi associate with in nature, and how they are affected by disturbance.

The field of plant ecology is somewhat ahead of that of ECM ecology because organisms that live mostly underground are difficult to study. However, recent advances in molecular biology have allowed researchers to characterize below-ground ECM communities more accurately and more easily. As a result, ECM community ecology is increasingly being related to plant and soil ecology to provide more thorough understanding of ecosystem structure and function.

Literature Review

Fungal Identification

Morphological Methods

ECM fungi are most readily identified by their sporocarps (sexual reproductive structures). Although fungal ecology studies based on sporocarps provide valuable information, they are not always representative of ECM fungal community structure on root tips (Jonsson *et al.*, 1999b; Durall *et al.*, 1999; Gardes & Bruns, 1996; Visser, 1995). The advantage to observing the ECM community on root tips is that one can directly link each fungal species with its host plant, which is particularly helpful in mixed stands. Agerer (1987) and Ingleby *et al.* (1990) produced the first comprehensive guides for identification of ECM fungi based on morphology and anatomy of mycorrhizal root tips. Their identification was based on tracing fungal mycelia from sporocarps to root tips, and (or) by regular co-occurrence of particular sporocarp species and ECM root tip morphotypes. These guides are useful for identifying most ECM root tips to family or genus, and in the few cases where morphology is particularly distinctive, to species.

Many useful studies have used morphotyping exclusively to characterize below-ground ECM communities in nature and in bioassays (e.g. Kranabetter, 1999; Durall *et al.*, 1999; Goodman & Trofymow, 1998a; Harvey *et al.*, 1997). However, morphotyping alone is usually limited in identification to ECM fungal species. This is partly because of the wide range of root tip morphology displayed by a single ECM species on different hosts, in different environments, and at different developmental stages. Furthermore, the suite of morphological characters available for examination on ECM root tips is often inadequate to discern species within difficult taxonomic groups, such as *Cortinarius*, *Inocybe*, *Russula*, and *Tomentella*, all of which are important ECM genera. In diverse ECM systems, molecular methods must be employed to accurately determine the taxonomic placement of ECM fungi. Another more recent guide to ECM descriptions (Goodman *et al.*, 1996) incorporates molecular identification information in addition to detailed morphological descriptions.

Molecular Methods

Since the early 1990s, researchers have been identifying fungi by using PCR to amplify known regions of fungal DNA from ECM root tips. The most commonly amplified region is the internal transcribed spacer (ITS) region, consisting of two ITS segments between the nuclear DNA coding for 18s, 5.8s, and 28s ribosomal subunits. While combined analyses of other DNA loci are often necessary to

establish well-supported phylogenies at higher taxonomic levels (Frøslev *et al.*, 2005; Bruns & Shefferson, 2004), the ITS region is adequate to distinguish species by RFLP or sequence analysis because of high interspecific and low intraspecific variation (Horton & Bruns, 2001; Kårén *et al.*, 1997). Horton & Bruns (2001), however, note that taxonomic affinity of fungi on ECM roots often remains unknown in studies using RFLPs because central databases for sporocarp RFLP patterns are not available and a variety of primers are used to amplify the region. Furthermore, there are several examples of different species within a genus giving the same RFLP pattern (Durall *et al.*, 2006; Peter *et al.*, 2001b; Kårén *et al.*, 1997), as well as different RFLP patterns coming from a single species due to a single nucleotide difference in a restriction enzyme binding site (Kretzer *et al.*, 2003b). Matching RFLP patterns is also sometimes difficult due to error associated with resolution of agarose gels (Dickie *et al.*, 2003).

Recent studies have used RFLPs or T-RFLPs of the ITS region to group samples first, then sequence unique RFLP types to determine taxonomic affinity (e.g. Horton *et al.*, 2005; Cline *et al.*, 2005; Izzo *et al.*, 2005; Tedersoo *et al.*, 2003; Sakakibara *et al.*, 2002). It is common practice to accept that a similarity of at least 97% or 98% between an unknown sample sequence and a database (e.g. GenBank) sequence constitutes a species-level match. Izzo *et al.* (2005) and Cline *et al.* (2005) have also recently used ITS DNA sequences to group unknown genotypes of multiple ECM root tip samples with each other using sequence similarity or phylogenetic trees.

ECM Ecology

ECM and Disturbance

Response of forest ECM communities to disturbance has been a central topic in recent years. Many researchers have examined how ECM communities differ between young and mature forests, or how they are affected by natural disturbances or forest management practices. Clearcutting has consistently resulted in dramatic changes to ECM community composition and structure (Jones *et al.*, 2003). Wildfire effects have been weaker where fires are patchy and of low intensity (Jonsson *et al.*, 1999b), but intense stand-replacing fires can change community composition radically (Grogan *et al.*, 2000). Fire may especially reduce ECM fungi proliferating in the forest floor (Stendell *et al.*, 1999), but some species may readily recolonise after wildfire because they have resistant spores or vegetative propagules (Taylor & Bruns, 1999; Baar *et al.*, 1999). These propagules may successfully colonise post-fire because of their resistance to heat and desiccation, or because they tend to be distributed lower in the soil profile and thus avoid heat from fires.

Two important factors that contribute to ECM colonisation of new hosts after disturbance are spatial extent of the area disturbed and distance of new hosts from mature trees with established ectomycorrhizae. Durall *et al.* (1999) found a marked decrease in ECM sporocarp richness with increasing size of cutblocks, particularly larger than 900 m^2, but this decrease was not mirrored as strongly in the below-ground community. Hagerman *et al.* (1999b) found no effect of cutblock size on below-ground ECM communities. Studies show, however, that ECM diversity decreases and (or) community structure changes drastically with increasing distance from intact forest (Hagerman *et al.*, 1999a; Durall *et al.*, 1999) or isolated mature trees left after logging (Cline *et al.*, 2005; Kranabetter, 1999). This distance effect, in addition to seedling isolation effects (Simard *et al.*, 1997b; Fleming, 1984) suggests that vegetative parts of ECM fungi attached to live trees are important inoculum sources. However, there is also evidence that soil biology and chemistry contribute to differences between ECM communities in mature and recently disturbed stands (reviewed in Jones *et al.*, 2003).

ECM and the Soil Environment

The biotic and abiotic complexity of the soil environment is astounding, and physiological processes and interactions that occur underground are difficult to study. Nutrient cycling is a critical ecosystem function that is dependent on many factors including amounts and types of available substrates (e.g. Prescott *et al.*, 2000; Frazer *et al.*, 1990), soil moisture and pH (e.g Barg & Edmonds, 1999), soil microbial community composition (e.g. Houston *et al.*, 1998), and soil fauna (e.g. Forge & Simard, 2000).

ECM fungi play a key role in nutrient cycling by transforming and translocating chemicals in the soil. While transfer of available mineral nutrients from soil to plants is well known (see Smith & Read, 1997), the ability of ECM fungi to acquire nutrients from soil organic compounds has been explored more recently (Read & Perez-Moreno, 2003). Generally, ECM fungi are important to plant nutrition when available nutrients are limiting, but ECM colonisation and diversity decrease when nutrients are readily available (Jones & Smith, 2004). Amounts and forms of nutrients vary widely with forest type, making it difficult to generalize about ECM functions, but nitrogen is usually limiting in northern coniferous forests.

Some of the factors that control nutrient cycling also help determine ECM community structure. For instance, higher diversity of available substrates may result in higher ECM diversity. While some fungi prefer decaying wood (Smith *et al.*, 2000; Goodman & Trofymow, 1998b), others have affinity to particular forest floor layers or mineral soil horizons (Rosling *et al.*, 2003; Nelville *et al.*, 2002). Soil

moisture (O'Dell *et al.*, 1999; Gehring & Whitman, 1994) and available soil nitrogen (Avis *et al.*, 2003; Lilleskov *et al.*, 2002; Peter *et al.*, 2001a) also affect ECM community diversity and composition above- and below-ground over long gradients.

Plant Community Dynamics and Ectomycorrhizae

Plant communities in ECM forests are constantly changing because of variable disturbances, spatial structure, and plant competition. This state of flux has implications for ECM fungi. First, occurrence of tree hosts change, as do tree sizes and availability of roots. The two common models of plant succession, "relay floristics", where species groups establish sequentially over time, and "initial floristics", where all species establish soon after disturbance, are evident to varying degrees in temperate forests (Oliver & Larson, 1996). The pattern of succession that occurs in a given forest depends on pre-disturbance conditions, disturbance type, environmental conditons, and plant physiology and tolerances to environmental factors (Agee, 1993). Availability and dispersal of propagules are also important. Parallel models of ECM succession in forests have also been proposed, as discussed in detail in Chapter 2.

Study Overview, Objectives, and Hypotheses

This study was conducted in seral stands of paper birch and Douglas-fir the Interior Cedar-Hemlock biogeoclimatic zone (ICH) of southern interior British Columbia. The ICH zone is characterized by mild winters, when most of the precipitation falls as snow, and warm summers with scattered rains in May-June and sometimes in August. Seasonal drought can occur during July-August. Clearcutting has been the most common cutting method since the 1970's, but variable retention has been applied extensively over the past decade. Up to 13 conifer and broadleaf tree species grow in mixture in the ICH zone.

The overarching goal of my research was to re-evaluate existing theories concerning successional roles of ECM fungi. To do this, I studied ECM communities along a chronosequence of mixed stands of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and paper birch (*Betula papyrifera* Marsh.). I also examined relationships between ECM and site attributes, and compared wildfire with clearcutting effects on the ECM community. The specific hypotheses tested in this study were based on previous chronosequence studies of forest ECM fungal communities, studies of the effects of disturbances on ECM fungal communities, and studies of relationships between ECM fungal communities and environmental attributes. The following study objectives and hypotheses, along with their associated backgrounds and contexts, are addressed in Chapters 2 and 3:

OBJECTIVE 1: To characterize successional patterns of the below-ground ECM community with increasing age of mixed Douglas-fir — paper birch forests (Chapter 2)

- **HYPOTHESES**

1. ECM fungal species richness, diversity and evenness increase with stand age.
2. ECM communities and diversity differ between clearcut and burned forests.
3. Previously described "early stage" fungi (those forming E-strain mycorrhizae, and *Thelephora terrestris*) decrease in abundance and frequency with increasing stand age, while "late stage" fungi (*Cortinarius*, *Piloderma*, and *Russula* spp.) increase, and "multi stage" fungi (*Cenococcum geophilum* and *Inocybe* spp.) have no distinctive pattern.
4. The proportion of the ECM community comprised of ECM fungi shared between Douglas-fir and paper birch decreases with increasing stand age.

OBJECTIVE 2: To examine relationships between soil properties and ECM fungal community measures along a chronosequence of mixed forest stands that were similar in vegetation composition and site quality (Chapter 3)

- **HYPOTHESES**

1. Soil N and P availability and mineralizable N decrease with stand age, while the C:N ratio increases
2. Soil variables explain a substantial degree of variation in ECM diversity that is not accounted for by stand age; namely, inorganic N and P availability are negatively correlated with ECM fungal diversity, while organic P and C:N ratio are positively correlated
3. These soil variables are related to ECM community composition and structure.

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Chapter 2 Below-ground ectomycorrhizal community succession on Douglas-fir (*Pseudotsuga menziesii*) and paper birch (*Betula papyrifera*) in southern interior British Columbia[1]

Introduction

Ectomycorrhizal symbioses are ubiquitous in northern forest ecosystems and essential for tree host productivity. Because reciprocal feedbacks occur continuously between ectomycorrhizal (ECM) fungal species and tree hosts, ECM fungal species composition patterns should integrate with floristic succession of forested stands. In British Columbia, forested landscapes are frequently disturbed by fire, insects, disease, wind and humans, resulting in a dynamic mosaic of stands. Patterns of above-ground vegetation succession following wildfire and clearcutting have been fairly well characterised in forested systems (Oliver & Larson, 1996; Agee, 1993; Shugart, 1984), but patterns in ECM fungal succession have not (Jones *et al.*, 2003).

ECM fungal succession following afforestation by birch was first described in Britain over two decades ago, and ECM fungi were categorized as either "early-stage" or "late-stage", depending on infection timing, year of fruiting, and other fungal characteristics (Fleming, 1984; Mason *et al.*, 1983; Deacon & Donaldson, 1983; Fleming, 1983; Fox, 1983). However, this work was performed in young plantations on agricultural soils, which were not comparable to natural stands. Researchers continue to use "early-stage" and "late-stage" terminology (Bergemann & Miller, 2002; Visser, 1995). For example, some specific "early-stage" fungi, such as fungi forming E-strain mycorrhizae, fungi in the MRA complex, *Thelephora terrestris*, and *Amphinema byssoides*, dominate seedlings regenerating soon after disturbance, (Kranabetter, 2004; Hashimoto & Hyakumachi, 2000; Jones *et al.*, 1997). "Early-stage" and "late-stage", however, are general descriptive terms that do not adequately reflect the complexity of fungal succession patterns in ecosystems. Nonetheless, describing ECM succession patterns in relation to their environments may be fundamental to understanding their life-history strategies and functions.

[1] A version of this chapter will be submitted for publication. Twieg BD, Durall DM, Simard SW, Jones MD. Below-ground ectomycorrhizal community succession on Douglas-fir (*Pseudotsuga menziesii*) and paper birch (*Betula papyrifera*) in southern interior British Columbia.

Chronosequences representing forest succession after wildfire or clearcutting have recently been used to study ECM fungal sporocarp and root-tip communities (Kranabetter *et al.*, 2005; Smith *et al.*, 2002; Visser, 1995). Although effects of stand age on ECM diversity varied among these studies, some general trends in ECM fungal species composition and community structure have emerged. In North American forests, for example, "multi-stage" fungi (e.g. *Cenococcum geophilum*, *Inocybe* spp.) that occur in all stand ages have been described (Visser, 1995). As stands age, "multi-stage" fungi are augmented, rather than replaced, by a community of several "late-stage" fungi (e.g., *Cortinarius* spp., *Russula* spp., *Piloderma byssinum*, and *Tricholoma* spp.). In the jack pine forests studied by Visser (1995), only a few "early-stage" fungi (*Thelephora* and fungi forming E-strain mycorrhizae) were present in young stands but did not occur in older stands. Smith *et al.* (2002) found no strong differences in cumulative species richness of ECM sporocarps among stand ages, but observed increases in sporocarp biomass of *Cortinarius, Ramaria, Russula, Elaphomyces*, and *Rhizopogon* with increasing stand age. Kranabetter *et al.* (2005) found increased ECM sporocarp richness with western hemlock forest age, including increased frequency of some *Cortinarius* and *Russula* species, as well as overall increased richness of *Tricholoma* and *Craterellus* species. Visser (1995) and Kranabetter *et al.* (2005) both found that ECM fungal diversity was low in young, open stands but was significantly higher in the youngest stand ages sampled that had closed canopies. Both studies also found diversity did not change much with further stand development.

Host specificity of ECM fungi is also important in the succession of mixed-ECM host forests. Generalist fungal taxa comprise substantial proportions of the ECM community in mixed coniferous/broadleaf forests (Kennedy *et al.*, 2003; Molina *et al.*, 1992). The same is true in forests with two conifer hosts (Cullings *et al.*, 2000; Horton & Bruns, 1998). Older stands may, however, accumulate host-specific fungi with age, and some researchers believe that climax tree species associate with host generalist fungi to the greatest degree during their establishment period because it is important for them to achieve maximal colonisation by fungi present in the existing mycorrhizal networks of seral tree species (Horton *et al.*, 2005; Kropp & Trappe, 1982). Where the dominant tree species in a forest are infected by a high proportion of generalist fungi, there exists good potential for common mycorrhizal networks (CMNs) to connect trees underground (Simard *et al.*, 2002). These CMNs can facilitate carbon transfer between tree species, possibly moderating interspecific competitive interactions (Simard *et al.*, 1997a).

Certain ECM fungi appear to require established connections to trees in order to colonise new hosts (Hagerman *et al.*, 1999b; Kranabetter, 1999; Durall *et al.*, 1999; Kranabetter & Wylie, 1998; Simard *et al.*, 1997b; Fleming, 1984). This may be particularly true for fungi forming mycelial strands and/or rhizomorphs, which appear to function in vegetative spread (Cline *et al.*, 2005; Agerer, 2001; Newton,

1992). Several studies have shown that conspecific mature trees contribute to the establishment of seedling ECM communities (Jonsson *et al.*, 1999a; Kranabetter, 1999). Some shrub and herbaceous perennials also harbour ECM fungal species common to coexisting conifer hosts, contributing to the establishment of their ECM communities (Dickie *et al.*, 2004; Hagerman *et al.*, 2001; Horton *et al.*, 1999). Jones *et al.* (1997) found a high proportion of shared fungi on one and two-year-old Douglas-fir and paper birch seedlings, and Simard *et al.* (1997c) found that the richness of shared fungi on one-yr-old Douglas-fir increased when it was grown in mixture with paper birch. How patterns of colonisation by host-specific and host-generalist ECM fungi change with stand age in succeeding mixed conifer/broadleaf forests has yet to be studied.

Ectomycorrhizal communities have been well studied on conifer seedlings regenerating within ten years of a stand-replacing disturbance, such as wildfire or clearcutting. These studies show that ECM fungal community composition differs between regenerating and mature stands, even though species diversity and colonisation often do not differ (Jones *et al.*, 2003). Few ECM community comparisons have been made between stands of different disturbance histories. Lazaruk *et al.* (2005) found fewer active roots in the soil immediately following burning of a *Picea glauca* (Moench) Voss stand than after clearcutting, as well as lower ECM fungal species richness and diversity, but these differences were slight. Mah *et al.* (2001) also found ECM fungal community composition differences between clearcut versus clearcut and broadcast burned sites, but no differences in diversity or colonisation.

The objective of this study was to characterize successional patterns of the below-ground ECM community with increasing age of mixed Douglas-fir — paper birch forests, and to improve on earlier studies of ECM succession by using molecular identification of ECM fungi and a well-replicated study design. The hypotheses addressed were: 1) ECM species richness, diversity and evenness increase with stand age; 2) ECM communities and diversity differ between forest of clearcut and wildfire origin; 3) previously described "early stage" fungi (E-strain-forming fungi and *Thelephora terrestris*) decrease in abundance and frequency with increasing stand age, while "late stage" fungi (*Cortinarius*, *Piloderma*, and *Russula* spp.) increase, and "multi stage" fungi (*Cenococcum geophilum* and *Inocybe* spp.) have no distinctive pattern; and 4) the proportion of the ECM community comprised of ECM fungi shared between Douglas-fir and paper birch decreases with increasing stand age. These hypotheses were tested by morphological and molecular characterization of ECM communities across a chronosequence of mixed Douglas-fir — paper birch forests in southern interior British Columbia.

Materials and methods

Site description and study design

The study sites were located in the Thompson Moist Warm (ICHmw3), Shuswap Moist Warm (ICHmw2), and Thompson Moist Cool (ICHmk2) Interior Cedar-Hemlock variants of southern interior British Columbia (Lloyd *et al.*, 1990). Following wildfire, Douglas-fir, paper birch and lodgepole pine (*Pinus contorta* var. *latifolia* Doug. Ex Loud.) dominate seral ICH forests to approx. 120-150 years. Shade tolerant conifers establish immediately post-disturbance or slowly ingress with time (Agee, 1993), and form climatic climax stands comprised of western redcedar (*Thuja plicata* (Donn ex D. Don) Spach) and either western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) in the moist warm variants or hybrid spruce (*Picea engelmannii* Engelm x *Picea glauca* (Moench) Voss) in the moist cool variants (Lloyd *et al.*, 1990). Maturing seral stands of Douglas-fir are often logged at between 120 and 150 years of age in the study area.

Single cohort stands were selected to represent a chronosequence following stand-replacing fire and clearcutting. Four stand age classes were chosen to represent important stages (Oliver & Larson, 1996) of mixed stand development: 4- to 6-years-old (stand initiation stage, where Douglas-fir naturally seeds in or is planted, and paper birch naturally seeds-in or sprouts from existing root stocks), 21- to 30-years-old (canopy closure followed by stem exclusion stage), 60- to 70-years-old (stand re-initiation stage, where birch starts to die out of the stand, creating canopy gaps) , and 90- to 103-years-old (stand re-initiation stage, where Douglas-fir is dominant over remaining paper birch). For brevity, these age classes will be referred to as 5-, 26-, 65-, and 100-yr-old stands, respectively, and stands of wildfire origin will be referred to as burned. For the two youngest age classes, clearcut and burned stands were selected, but only burned stands were used for the oldest two age classes. Clearcut stands in the two oldest age-classes were not available in the study area, precluding a complete factorial study design. Four replicate sites were selected for each of the six stand (age class/initiation-origin) types.

The only sites not located in the ICHmw variants were two 26-yr-old burned sites, which were located in the ICHmk2 variant due to difficulty finding appropriate sites in the ICHmw variants. All burned sites of 20-years-old and older naturally regenerated to Douglas-fir and paper birch, whereas clearcut sites and 5-yr-old burned sites had been planted to Douglas-fir with natural regeneration of paper birch. Paper birch trees from both seed and stump-sprout origin were present at both clearcut and burned 5-yr-old sites. Stand ages were determined from B.C. forest cover maps (B.C. Ministry of Forests; Victoria, B.C., Canada) and, for naturally regenerated sites, by coring five Douglas-fir trees. For

plantations in the youngest age class, age represents the number of years since planting; naturally regenerated Douglas-fir were absent from these stands.

Site selection was based on the following criteria: 1) Douglas-fir and paper birch comprised at least 75% of the total canopy cover of ECM host tree species in a 40 m by 40 m area (Table 2.1), 2) moisture regime was submesic to mesic and site series was zonal (Lloyd *et al.*, 1990), and 3) distance to other replicate stands was at least one kilometre. Careful excavation of Douglas-fir seedlings prior to sampling in 5-yr-old sites showed that their root systems rarely extended beyond 40 cm of their bole, and tracing of other conifers' root systems outward indicated little chance that their root systems overlapped those of the Douglas-fir seedlings. Once all sites were selected, one 30 m by 30 m plot was established at each site. Buffer zones of 10 m (5-yr-old sites), 15 m (26-yr-old sites) 25 m (65- yr-old sites), and 30 m (100-yr-old sites) were delineated to eliminate edge effects. Midpoints of plot edges were marked to delineate quadrants.

Sampling for Ectomycorrhizae

Soils were sampled for ectomycorrhizae twice in 2004: late May to early June (spring) and late September to early October (fall). For each sampling period at each site, eight separate pairs of trees, including one Douglas-fir and one paper birch each, were selected inside the plot (two each per plot quadrant). The maximum inter-tree sampling distance for greatest likelihood of obtaining roots from both tree species was 3 m in 5-yr-old stands, 5 m in 26-yr-old stands, 7.5 m in 65-yr old stands, and 10 m in 100-yr-old stands. One soil sample was taken from within a 0.5 m-wide transect between the two trees in each pair (one sample x eight locations x 2 sampling seasons = 16 samples taken per site). In 5-yr-old sites, samples were taken within 30 cm of the Douglas-fir boles due to the limited radial extent of their root systems, and preliminary samples showed ample birch roots were also available in these sampling locations. In all other age classes, samples were taken at the midpoint between the two trees of each pair. The minimum distance between any two sampling locations was 2 m.

Soils were sampled far enough from other ECM host trees that there was little chance that soil samples included their roots. This was an issue mostly in 26-yr-old stands, where black cottonwood (*Populus trichocarpa* Torr. & Gray), trembling aspen (*Populus tremuloides* Michx.), willow (*Salix*) spp., and hybrid spruce occurred in the canopy, and western hemlock occurred in the understorey. Where sampling locations were less than 5 m from these trees, their presence was noted. At each sampling location, forest floor and mineral soils were removed in a 9 cm X 9 cm area using a machete and trowel (soils were too stony to use corers). The forest floor depth was recorded, and the LFH layers placed

together in a plastic bag. Mineral soil was then removed to 20 cm depth from the bottom of the forest floor and bagged separately. Samples were stored on ice in a cooler until transfer later the same day to a 4 °C walk-in cooler.

Soil samples collected in the spring from 5-yr-old burned sites yielded insufficient root tips. For fall sampling, three Douglas-fir seedlings were therefore removed from plot buffer zones in these sites and 5-yr-old clearcut sites. Seedlings were excavated to a depth of 30 cm and 25 cm radius around the bole, their stems removed, and the soil blocks transported in garbage bags the same day to the 4° walk-in cooler. Core-type soil samples and seedlings were taken in fall from 5-yr-old clearcut sites, but only seedlings were taken from 5-yr-old burned sites. This meant that 5-yr-old burned stands could be compared statistically only to 5-yr-old clearcut stands, and only based upon the ECM communities of the excavated Douglas-fir seedlings.

Sorting of Root Tips

One soil sample from one site of each stand type was processed in a rotation, with the site changed in each successive rotation to mitigate potential confounding effects from samples remaining in the cold room for different amounts of time. Forest floor (when present) and mineral soil samples were processed separately. Forest floor samples were first soaked in distilled water for a few hours. All samples were washed gently with tap water over 4-mm and 2-mm stacked sieves. All woody and fine roots were removed with forceps from both sieves and placed in distilled water in a glass baking dish. Root segments were cut into 1.5 cm segments and gently mixed, and obvious ECM tubercles were sliced in half due to their clumpiness. Segments were randomly selected over a numbered 2 cm grid for viewing under a dissecting microscope.

Viability of root tips was determined based on colour and turgidity (Harvey *et al.*, 1976), and by whether or not the stele was intact and not easily broken. Ectomycorrhizal status was determined by the presence or absence of a fungal mantle. For tips lacking an obvious mantle but showing ECM characteristics (e.g., inflated shape, branching, lack of root hairs), a representative was examined for presence of a Hartig net at 400X magnification under a compound microscope. Douglas-fir and paper birch roots were differentiated by the size of root tips and, to a lesser extent, by colour and texture of larger roots. When these characteristics were ambiguous, ECM cross sections were viewed under a compound microscope. Mycorrhizal paper birch root tips were differentiated from Douglas-fir by their radially elongated epidermal cells. It is possible that roots of other broadleaf trees were counted as birch roots and those of other conifer species counted as Douglas-fir, so error in host tree identification was

estimated from root tips using molecular methods. Primers trnLc and trnLd were used to amplify host DNA from the same root tip samples used for ECM fungal identification, and PCR products were digested with *Hinf* I and *Taq* I as described in Brunner *et al.* (2001). Ten samples of Douglas-fir root tips and fifteen samples of paper birch root tips were identified in this manner from samples taken within 5m of other ECM host trees, and fifteen more samples of each host species were identified from randomly selected samples.

Successive root segments were examined until 50 live Douglas-fir and 50 live paper birch root tips were counted from each soil layer per sample. Percent ECM colonisation was determined from the ratio of live mycorrhizal to total live tips. Segments with ectomycorrhizae were then further cut into 5-10 mm segments (without cutting ECM tips) and 25 tips were randomly selected from a 5 mm grid for morphotyping (2 hosts x 2 soil layers x 25 tips =100 root tips per soil sample). Where less than 50 root tips of a particular host occurred in a soil sample, root tips of the other host species were sampled to bring the total number of ECM tips to 100 per soil sample. Similarly, if one soil layer from a soil sample did not contain enough roots, or if no forest floor layer was present for that sample, then additional roots were sampled from the other soil layer.

Roots of seedlings from 5-yr-old sites were washed, clipped from the bole, cut into 1.5 cm pieces, and randomly sampled until 400 root tips were counted and classified as mycorrhizal or non-mycorrhizal. Morphotypes were determined for 200 randomly selected tips. Paper birch roots present in the samples were similarly subsampled and morphotyped where available.

Morphological and Molecular Identification of ECM Root Tips

Morphotyping

All subsamples were examined according to Goodman *et al.* (1996). Branching pattern, system and root tip sizes, mycelial strands, and emanating hyphae were examined under a dissecting microscope. Emanating elements and inner and outer mantle patterns of mantle peels were examined at 400X and 1000X under a compound microscope. Chemical tests were used for taxonomic affinity to family or genus as described in Kernaghan *et al.* (2003) and Agerer, (1987). Notes were compiled on colour, branching pattern, system dimensions, emanating elements, cell dimensions, mantle patterns, hyphal junctions and anastomoses, cystidia, etc., and photographs were taken of important features (Appendix A) using a DMX 12000 digital camera mounted to a SMZ 1000 dissecting microscope and Eclipse 800 compound microscope outfitted with Differential Interference Contrast (Nikon, Melville, New York, USA). Up to three subsamples, where available, per morphotype per host in each soil sample were set

aside for potential molecular identification. For each subsample, 1-5 root tips were placed in 100 µl of sterilised millicue water in a microcentrifuge tube, freeze-dried, and stored at -80°C until DNA extraction. Subsamples of morphotypes were neither mixed within a soil sample nor between soil samples. Soil samples were processed over six months for each sampling period.

DNA Extraction and PCR Amplification

One subsample per morphotype per soil sample was subject to DNA extraction and PCR amplification of the fungal ITS region of nuclear rDNA. About 540 of these samples had ITS regions DNA sequenced. In addition, 2-3 samples each of 15 morphotypes were analysed by RFLP or DNA sequencing to check accuracy of within-soil sample morphotype sorting. Seven of these 15 morphotype selections contained subsamples from both host species in the same soil samples.

ECM root tip samples were pulverized in 400 µl of CTAB buffer (3% CTAB; 100 mM Tris-Cl pH 8.0; 1.4 M NaCl; 20 mM EDTA; 2 % PVP; 0.2% β-mercaptoethanol) with a ceramic bead in a FastPrep beater machine (Qbiogene, Irvine, CA, USA) for 45 sec. and were then incubated at 65°C for 90 minutes. DNA was isolated with two repetitions by adding of an equal volume of chloroform-isoamyl alcohol (24:1), mixing by inverting for one minute, centrifugation at 13 000 rpm for 10 min., and removal of the aqueous phase. DNA was precipitated overnight in 2/3 volume of isopropanol, followed by two washes with 70% ethanol. The pellet was dried in a speed-vac for 10 minutes, and resuspended in 100 µl of low-EDTA TE buffer (10 mM Tris-Cl pH 8.0; 0.1 mM EDTA).

PCR reactions were carried out in 25 or 50 µl reactions with the following concentrations: 1.5 mM $MgCl_2$, 1.6 mg μl^{-1} BSA, 0.2 mM dNTP's, 0.48 µm each primer, and 0.25 units μl^{-1} of Ampli-Taq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA). Primers were synthesized by Nucleic Acid and Protein Services (NAPS) at UBC, Vancouver. Template DNA was added in the amount of 1 µl per 50 µl reaction. DNA was diluted tenfold for samples that did not amplify the first time. A PTC-200 Thermocycler (MJ Research Inc., Waltham, MA, USA) and a GeneAmp 2700 Thermocycler (Applied Biosystems) were used. An initial cycle of 10 min. at 95 °C was used to activate the polymerase, followed by 34 cycles of the following: 45 sec. denaturation at 94 °C, 45 sec. of annealing, and 1 min. of extension at 72 °C. A final extension step of 7 min. was added. Annealing temperatures varied with the primers and thermocyclers used. Optimum annealing temperatures were determined on the PTC-200 by running a gradient in 0.5 °C increments above and below the T_M calculated by NAPS, and on the GeneAmp 2700 by running the annealing temperature at 1 °C below the T_M determined by the thermocycler's calculator.

Several primer pairs were used to amplify fungal DNA from root tips. After many initial amplification attempts with primer NL6Bmun for intended RFLP analyses, it was determined that its success rate (about 50%) was unacceptable, and its use was discontinued. For samples slated for DNA sequencing, primers NSI1 and NLC2 (Martin & Rygiewics, 2005) were attempted first because they showed the highest success in a comparison of several primer pairs on a set of random samples. Initial results showed poor amplification of *Rhizopogon* and *Suillus* mycorrhizae with these primers. Subsamples fitting those morphotypes were thereafter amplified with ITS1f and ITS4 because these primers have been used successfully with these taxa before (Kretzer *et al.*, 2003b; Bruns *et al.*, 2002). Other basidiomycete morphotypes that did not amplify well with NSI1 and NLC2 were attempted with ITS1 and ITS4B (Gardes & Bruns, 1993).

PCR products were visualized on 1.5 or 2% agarose gels made with ethidium bromide. Gels were photographed with a Kodak Gel Logic® 440 gel documentation system. Single-band PCR products were cleaned with a Charge Switch PCR Cleanup Kit (Invitrogen, Carlsbad, CA, USA). For products that showed multiple bands and ample amounts of DNA, 20-40 µl of PCR product were separated on 1.5% gels. If bands were sufficiently far apart, they were excised from gels on a UV transilluminator, and their DNA was purified with a QIAquick Gel Extraction kit (Qiagen Inc., Valencia, CA, USA).

RFLP Analysis

It was decided that using RFLPs as the main method for grouping samples would be inadequate because of the problems discussed in Chapter 1. Therefore, RFLPs of the fungal ITS region were used in this study mainly to check on accuracy of within-soil sample morphotype sorting. Primers ITS1 and NL6Bmun were used to amplify DNA for these RFLPs. Several tuberculate *Rhizopogon* samples were also checked by amplification with ITS1f and ITS4B and restriction digestion with *Alu* I, as Kretzer *et al.* (2003b) showed this to be an easy and effective way of distinguishing species of *Rhizopogon vinicolor*-like ECM. Restriction digests were performed on PCR products and RFLPs visualized on gels as described by Hagerman *et al.* (1999b). Kodak 1D software (Kodak Instruments, Rochester, New York, USA) was used to estimate fragment lengths, and GERM software to suggest matches within the acceptable error suggested by Dickie *et al.* (2003).

DNA Sequence Analysis

Samples amplified with primers NSI1 and NLC2 were sequenced with primers ITS1 and NLB4 (Martin & Rygiewics, 2005) using the Big Dye Terminator Kit (Applied Biosystems). Those initially

amplified with ITS1 and ITS4B were sequenced with ITS1 and ITS4. Sequencing was performed on a 3730S capillary sequencer (Applied Biosystems) at NAPS or on a 3130x1 capillary sequencer (Applied Biosystems) at UBC Okanagan Fragment Analysis DNA Sequencing Services (FADSS).

Forward and reverse sequences were aligned, manually corrected, and trimmed in Sequencher 4.2 (GeneCodes, Ann Arbor, MI, USA). Consensus sequences were BLAST searched (Altschul *et al.*, 1997) through NCBI (National Center for Biotechnology Information, 2006) and UNITE (http://unite.zbi.ee) (Kõljalg *et al.*, 2005) websites to suggest taxonomic affinities of the samples. A taxon was considered a proper species match to a root tip sample if their sequences had 98% or greater similarity and aligned over at least 450 base pairs. Samples that sequenced poorly in one direction were BLAST searched with a single-pass sequence. These samples were considered proper matches at 97% similarity or better due to error rates of single-pass sequencing (Izzo *et al.*, 2005). The number of bases aligned and percent similarity were checked for the ten highest-scoring matches. When matches were less than 98% (97% for single pass) and/or under 450 bp, it was deemed relatively unimportant which BLAST match was "best" by any objective measure, since these matches did not solely determine the taxonomic placement of samples. Preference was given to matches to identified sporocarps. If no proper species match was made to a sample, then the taxonomic placements of the ten top-scoring matches were checked. If they consistently fell in the same family or genus, then the unknown sample was placed into that group.

After general taxonomic placement of samples by BLAST searching, a separate multiple alignment file of root tip sample sequences and sporocarp sequences from Durall *et al.* (2006) was made for each of the following groups: *Cortinarius*, *Hebeloma*, *Inocybe*, *Lactarius*, *Piloderma*, *Russula* and non-*Lactarius* Russulaceae, and Thelephoraceae. Multiple alignments were performed in ClustalX (Thompson *et al.*, 1997) for each group using the IUB algorithm, and corresponding sequence similarity matrices were created in the DNADIST program in a current version (3.63) of PHYLIP (Felsenstein, 1989). Pairwise and multiple alignment gap penalty parameters were adjusted as suggested by Hall (2004), and the resulting alignments were visually compared for quality. Since the ITS region is so variable due to common indels (Horton & Bruns, 2001), biologically reasonable alignments usually resulted from setting gap penalties somewhat low (gap opening at a value of 12 and gap extension at a value of 1). Similarity calculations ignored gaps and missing data. The same criteria for species matching as used with BLAST searching were used to match unknown samples to each other. After matches were determined, multiple alignments were again scrutinised in order to detect potential spurious matches due to alignment flaws. Samples with ambiguous matching were excluded from analyses.

While the original intent was to obtain DNA information on one subsample per morphotype per soil sample, lack of time and funding necessitated that we use some morphological data along with DNA information for the final classification. No attempt was made to analyse molecular data for *Cenococcum geophilum*, because Sakakibara *et al.* (2002) confirmed with molecular methods (ITS region) that morphological identification was reliable for this species. Douhan & Rizzo (2005) also showed that genetic diversity was as high between *C. geophilum* samples from the same soil core as it was between samples from different parts of the U.S.A. They state that this multi-locus genetic diversity may signify sympatric cryptic speciation. Given limited project resources, it was decided that attempts to define different species in the *C. geophilum* complex would be impossible.

Data analyses

ECM from Soil Samples

Statistical analyses were carried out using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) unless otherwise noted. Least-squares means of percent ECM colonisation of Douglas-fir and birch were calculated for stand types, due to the unequal numbers of soil samples containing target root tips from different sites. Logistic regression was used to determine whether time of soil sample refrigeration affected molecular results. For this analysis, molecular "success" was defined as a sequence that was useable and consistent with the morphotype. "Failures" had consistent double-peaks, were matched with saprotrophic taxa, or were otherwise inconsistent with the morphotype.

Taxa-sample unit curves were created using the resampling procedure in PC-ORD version 4 (McCune & Mefford, 1995-2002) for evaluating whether the sample size was sufficient to characterize the community at each site. One set of curves represents the average of curves for the four replicates per stand type, and a second set was created by pooling the four replicates of each stand. Rank-abundance plots were also generated for each stand type and host, using stand type species abundance data pooled for the four replicates.

Separate calculations and analyses were performed for site-level ECM fungal abundance data (i.e. number of root tips colonised by each taxon) and frequency data (i.e. the number of soil samples in which each fungal taxon was found). Root tip abundance of ECM fungal taxa is more informative about community structure than their frequency alone. However, abundance may give a skewed representation of ECM community structure because of spatial patchiness of ECM species (Cline *et al.*, 2005). PC-ORD was used to calculate site-level species richness, evenness, and Shannon-Weaver and Simpson diversity (1-D, the complement of Simpson's original index) indices. These data were calculated for Douglas-fir

and paper birch ECM communities separately and for their combined communities. 1st and 2nd order jack-knife estimators of species richness were also calculated, based on the combined community, for each site.

Two-way analyses of variance (ANOVA) were used to test for mean differences in species richness, evenness, diversity (Shannon-Weaver and Simpson indices), and relative abundance of shared ECM fungal species (i.e., species on both hosts) among stand types (main treatment effect) and between host species (subplot effect) using a split-plot completely randomised design (CRD) (Table 2.2). One-way ANOVA for a CRD was used to compare the whole ECM fungal community (of both hosts combined) between stand types. PROC GLM was used for the ANOVAs and TDIFF for pairwise mean comparisons, with a Bonferroni adjusted significance level of $\alpha = 0.05$, unless otherwise noted. Normality was checked with PROC UNIVARIATE, using a $p \geq 0.05$ criterion for Anderson-Darling and Shapiro-Wilks tests. Homogeneity of variance was checked by examination of residual vs. predicted plots and by Bartlett's test.

Principal Components Analysis was performed on the multivariate dataset of species richness, evenness, Shannon-Weaver diversity, and Simpson diversity of the Douglas-fir, paper birch, and combined communities, plus the 1st order jackknife estimate for the combined community (i.e. 13 variables). Site scores for the first principal component were regressed, using least-squares, against all possible combinations of the following predictor variables that included at least one age variable: stand age, stand age squared, 1/stand age, initiation type (dummy variable) and subzone (dummy variable). Akaike's Information Criterion (AIC) adjusted for small sample size (AIC_C) was used to select the best model from the set of candidates (Burnham & Anderson, 2002).

Since the ICHmk has a different climax tree community and a shorter growing season than the ICHmw, regressions were performed on ECM diversity data of the Douglas-fir community and the combined community to check for effects of subzone. The same five predictor variables were included as were used in the regression of the diversity principal component. However, three model selection methods were used in PROC REG instead of AIC_C for each diversity variable: stepwise, backward, and forward. Significance of $\alpha = 0.1$ was used for the criterion of variable retention in models.

Sites were ordinated according to ECM fungal communities using nonmetric multidimensional scaling (NMS). Separate NMS ordinations were performed on abundance data and frequency data in PC-ORD using the Relative Sorensen distance measure. With frequency data, another ordination was done with species grouped at the genus level because some community trends can be more obvious at this

level. This is true because genera like *Russula*, *Cortinarius*, and *Tomentella* are very speciose and ECM communities are often comprised of many rare species and only a few, if any, frequently found species (Taylor, 2002). Ordinations with all three types of input data were run for the community on Douglas-fir, the community on paper birch, and their combined community. For these ordinations, "autopilot mode" was employed from a random starting configuration (McCune *et al.*, 2002). This mode chooses the best solution for each dimensionality (up to six) from 40 runs on the real data and provides a Monte Carlo test for significance of real data runs by comparing them to 50 randomizations on each dimension.

Abundance and frequency of particular ECM fungal taxa were examined more closely. These taxa were chosen because they had correlations of at least 0.6 (absolute value) to at least one of the two axes with the highest R^2 values from an ordination, or because they ranked as one of the ten fungal taxa of highest mean relative abundance in at least one of the stand types. Two statistical approaches were taken. First, mean relative abundances of taxa occurring on only one host were tested for stand type differences with one-way ANOVAs. Mean relative abundances of taxa that occurred on both hosts were also tested for differences among stand types and between host species using two-way (split-plot) ANOVA as above. Data not meeting normality assumptions were arcsine-square-root transformed; where this did not help, data were analysed using Kruskal-Wallis tests.

The second approach for examining individual species occurrence patterns was to use PROC GENMOD for modelling taxa abundance and frequency using count data (Cameron & Trivedi, 1998; McCullagh & Nelder, 1983). All possible combinations of the variables stand age, 1/stand age, subzone, and stand initiation type were used as predictors. The reciprocal of stand age was included as a predictor because earlier chronosequence studies (see Introduction) showed that relationships of ECM fungal abundances to stand age likely are not linear. Extra soil samples previously excluded from site-level analyses for removing sampling-effort differences between sites were included in abundance models because these models were based on abundance data from individual soil samples. All models were run with the Poisson and negative binomial distributions. While the Poisson distribution is often used to model count data, clumpy spatial distribution of species can result in data better fit to the negative binomial distribution (Krebs, 1999). Clumpy spatial distributions of ECM fungi within sites are well documented (Tedersoo *et al.*, 2003), but patchiness among geographically distinct stands is not well studied. Fit to the two distributions was compared as suggested by Cameron & Trivedi (1998). AIC was used to select the best model from abundance models, and AIC_C was used to select the best frequency models. Likelihood ratio tests of predictor variables were checked, and Type III tests were used in models with more than one predictor variable. Predicted values from abundance models were relativised to average root tips per core for the appropriate host(s).

ECM from Seedlings

Shannon-Weaver and Simpson diversity indices, as well as species richness and evenness were calculated for both initiation types of 5-yr-old stands based on abundance data from seedlings. Each of these four measurements, plus percent ECM colonisation, was tested for a difference between initiation types with two-tailed t-tests assuming equal variance. NMS ordinations were run with frequency and abundance data as described for the ECM community from soil samples.

Results

ECM from Soil Samples

ECM Colonisation and Distribution

Mean ECM colonisation was at least 97% for all stand types. This was based on the following average sample characteristics per site: 6 soil samples containing 50 root tips each of Douglas-fir and paper birch, 2 soil samples containing 100 root tips of only Douglas-fir, and 2 soil samples containing only 100 paper birch tips. One important exception was one 26-yr-old burned site that yielded only 5 soil samples with Douglas-fir root tips and one 65-yr-old site that yielded only 9 total soil samples with sufficient ECM root tips.

Molecular methods showed that identification of Douglas-fir root tips was correct for all of the 25 samples selected. However, for paper birch, 2 out of 15 samples taken within 5 m of other ECM broadleaf host trees gave RFLPs matching the genus *Salix* (Brunner *et al.*, 2001). One out of 15 samples taken more than 5 m from other ECM broadleaf host species also matched to the genus *Salix*. This genus had the greatest presence in 26-yr-old stands, with scattered occurrences in 5-yr-old stands and only one other occurrence in a 65-yr-old stand. However, the 15 samples checked accounted for 83% of the total examined samples that were taken within 5 m of another broadleaf species in 26-yr-old stands, so the overall error in birch identification was likely quite low.

Identification of ECM Fungi

On average, 476 Douglas-fir and 462 paper birch ECM root tips were identified per site with sufficient morphological and/or DNA support. Out of 541 DNA sequences analysed, 83% were of sufficient quality and length to place into genotypes, but 13% of these were identified by BLAST search as non-target, co-occurring fungi that were preferentially amplified in the PCR. About 30% of the non-target sequences were either from fungi in either the MRA complex (mostly *Phialocephala fortinii*) or matched fungal sequences amplified from roots of Ericaceous plants. Several samples that were obviously colonised by *Leccinum*, *Suillus*, and *Lactarius* spp. from morphotyping produced sequences that grouped well with *Rhizoctonia* or *Inocybe* spp. when amplified with ITS4B. Other non-target species included saprotrophic Ascomycetes and Basidiomycetes. The remaining 17% of sequences could not be analysed due to double sequence peaks. Logistic regression analysis showed that the probability of PCR products succeeding to produce useful target sequences was significantly negatively related to the amount of time their respective soil samples were stored in refrigeration. The predicted decrease in the

probability of success decreased from about 80% initially to 58% after samples had been stored for 6 months ($p = 0.02$ for likelihood ratio test for variable of time stored; AIC of model including time in storage variable= 649.9; AIC of model with intercept only = 653.3).

In total, 105 unique ECM genotypes (hereafter referred to as "species"), all with genetically-determined taxonomic affinity appropriate to their respective morphotypes, were used for analyses in this part of the study. The average useable sequence length was 706 base pairs (std. dev. = 148). Proper matches to query sequences that aligned over at least 450 bp were mostly unambiguous, with the exception of samples matching *Tomentella ramosissima* in the NCBI BLAST search, which also matched well to *T. lapida* in the UNITE BLAST search. In the genus *Cortinarius*, one sample gave a DNA sequence of 467 base pairs, but matched 98% or above with several obviously distinct *Cortinarius* genotypes from this study. This sample was thus excluded from analyses, and probably matched spuriously because the 467 base pairs included the whole 5.8s ribosomal DNA sequence. ECM fungal species and their corresponding BLAST search results and mean relative abundances by stand type are listed in Appendix B.

Generally, morphotypes deemed unique due to obvious macro- and microscopic characteristics were supported as being distinct species by DNA evidence. However, distinctions by morphotyping within and among the genera *Cortinarius* and *Hebeloma*, among most species of the genus *Russula*, and within the families Thelephoraceae and Sebacinaceae were not possible due to morphological similarity of different species. Therefore, only samples of distinctive morphotypes that were well-supported by DNA sequences, as well as samples that were sequenced, were included in analyses. Exceptions to this rule were two *Lactarius* morphotypes for which DNA amplification attempts were unsuccessful, but which had very distinct morphology and anatomy in comparison to other *Lactarius* species identified by molecular methods. All RFLPs and sequences of morphotype subsamples taken within the same soil samples indicated that within-soil sample morphotype groupings were accurate.

Rhizopogon vinicolor and *R. vesiculosus* (*sensu* Kretzer *et al.*, 2003b), tuberculate species difficult to distinguish by morphology, were encountered frequently. We could not analyse DNA from all observations of these sister species, but all DNA sequences that were analysed for this tuberculate morphotype matched one of these two species. Some ambiguity was encountered in the morphological features separating the species (Kretzer *et al.*, 2003b). Since both species were identified by DNA analysis for numerous observations in all stand types, they were lumped as *Rhizopogon vinicolor*-type for analyses. The NSI1-NLC2 primer pair seemed to preferentially amplify Ascomycetes co-inhabiting tuberculate *Rhizopogon* samples, as over 50% of sequences from these samples matched Ascomycete

sequences. There were also two cryptic species in the *Piloderma fallax*-like morphotype as determined by DNA sequence analysis, but their high frequency also did not permit sequence analysis of many samples. Hence, these two species were lumped as *Piloderma* spp. in the analyses.

ECM Community Diversity

Mean species-sample unit curves showed that sampling was inadequate for all stand types (Fig. 2.1a). Sampling inadequacy appeared least serious in the 5-yr-old clearcuts and 26-yr-old burned sites, but curves for the 26-yr-old burned sites were more similar to 26-yr-old clearcuts when the two burned ICHmk sites were removed from the analysis (Fig. 2.1b). The overall pattern of curves generated by grouping all four replicates for each stand type was similar to that of site-level curves (Fig. 2.1c). The rank abundance graph for the combined community shows that evenness was lower in the 5-yr-old clearcuts than the older types (Fig. 2.2a). In the Douglas-fir community, 5-yr-old stands displayed a much steeper abundance curve than older stands (Fig. 2.2b). Species abundance patterns in the paper birch community were similar among age classes (Fig. 2.2c).

Analyses of variance on richness, evenness, and diversity all detected significant stand type by host species interactions because differences among stand types were more extreme in the Douglas-fir ECM community than that of paper birch (see Table 2.3 for ANOVA results). Mean richness of the Douglas-fir ECM community was significantly lower in the 5-yr-old clearcuts than all other stand types except the 26-yr-old burned type (Fig. 2.3a). The two oldest stand types were also significantly richer than the 26-yr-old burned type. Richness on paper birch tended to be lower in 5-yr-old clearcuts than the other sites, and this difference was significant for 26-yr-old clearcut and 65-yr-old stand comparisons. The maximum difference in richness between stand types was much larger for Douglas-fir than birch, and the birch ECM community was significantly richer than the Douglas-fir community in 5-yr-old clearcuts and 26-yr-old burned sites.

ECM species diversity and evenness on Douglas-fir were lower in the 5-yr-old clearcuts than all other stand types, but these measures for paper birch did not differ among stand types (Figs. 2.3b-d). Diversity was about 3 times as high for paper birch as Douglas-fir in the 5-yr-old clearcuts. The jack-knife richness pattern for the combined community was similar to the patterns in Shannon-Weaver diversity for the Douglas-fir and combined communities (Fig. 2.4). Using frequency for diversity estimates yielded the same patterns as abundance (Table 2.4).

The first principal component representing a combination of species diversity variables accounted for 74% of the total variation. The best AIC_C-selected regression model of site scores for this principal component included 1/stand age and subzone as predictor variables ($R^2 = 0.65$; both predictors significant at $\alpha = 0.05$). It predicted a sharp difference between 5- to 26-yr-old stands, and slight differences with among the older ages (Fig. 2.5). Subzone was significant (at $\alpha = 0.1$, sometimes at $\alpha = 0.05$) in regression models predicting species richness and diversity indices, except for evenness of the Douglas-fir or combined communities, and Simpson's diversity of the Douglas-fir community. Adjustment for subzone in the models removed the difference between clearcut and wildfire initiation types.

Of the 105 ECM fungal species observed in soil samples, 42 occurred on both hosts (i.e., were shared ECM fungi), 23 occurred only on Douglas-fir, and 40 only on paper birch. Overall mean relative abundance of shared ECM fungi was significantly higher on paper birch than Douglas-fir (by 75%). Shared ECM fungi on Douglas-fir were over five times more abundant in 65- and 100-yr-old stands than 5-yr-old stands (Fig. 2.6).

ECM Community Composition and Structure

Cenococcum geophilum was the most frequently encountered species, followed by *Rhizopogon vinicolor*-type, *Piloderma* spp., and *Leccinum scabrum*. *R. vinicolor*-type was the most abundant ECM type on Douglas-fir in every stand type (mean relative abundance was 23-82%). Paper birch root tips were dominated (in terms of abundance) by *C. geophilum*, except in 5-yr-old clearcuts, where *Lactarius pubescens* was most abundant, and in 100-yr-old stands, where *Piloderma* was dominant. Second-most dominant on Douglas-fir was *Rhizopogon rudus* in 5-yr-old clearcuts, *Suillus lakei* in both 26-yr-old stand types, and *Piloderma* in 65- and 100-yr-old stands. Although 22 species in Thelephoraceae were found, most were rare, and their combined relative abundance in all sites averaged only 5%. However, seven species in this family occurred at one 100-yr-old site and totalled 20% relative abundance at that site. *Amphinema byssoides* and MRA mycorrhizae were found in all stand types, but were relatively infrequent and low in abundance. *Laccaria* spp. were found only in 5-yr-old stands, but were neither frequent nor abundant.

All NMS ordinations were significant by Monte Carlo test except for the ordination based on ECM fungal species abundance on Douglas-fir. All ordinations were best represented by three axes, of which two explained 59- 85% of the total variation. Correlations of species to axes and R^2 values of axes are summarized in Appendix C. In all ordinations, stand age was well correlated with at least one of the two axes that had the highest R^2 values. These ordinations generally showed strong grouping of 5-yr-old

sites along both axes, while the older sites were more diffusely positioned along axes to which age was not a strong correlate (Figs. 2.7a-c, 2.8a-c, and 2.9a-c). The ordination based on ECM species frequency on Douglas-fir showed better grouping of sites by stand type than did the ordination based on ECM species abundance. *Russula* and *Piloderma* were consistently strongly correlated, and in the same direction, to axes to which stand age was well correlated. *Rhizopogon vinicolor*-type, *Rhizopogon* as a genus, *Leccinum scabrum*, and *Lactarius pubescens* were consistently correlated to axes in the opposite direction to which stand age was correlated.

Cenococcum geophilum relative abundance was unaffected by stand type, but was about four times higher on paper birch than Douglas-fir (17% vs. 4.4%). This difference was significant only in 26-yr-old clearcuts and 65-yr-old burned stands (Fig. 2.10a). Mean relative abundance of *Russula* spp. did not differ between host species, but was higher in the oldest than all other stand types, except the 65-yr-old stands (Fig. 2.10b). The 100-yr-old stands had eighteen times more *Russula* than 5-yr-old stands, and three times more than 26-yr-old stands. *Russula* was also more abundant in 65- than 5-yr-old stands. *Lactarius scrobiculatus* was more abundant on paper birch than Douglas-fir (6.2% vs. 1.3%, respectively, particularly at the 26-yr-old burned sites) (Fig. 2.10c). Relative abundance of *Cortinarius* and *Piloderma* spp. differed among stand types (Figs. 2.10d and e, respectively), but that of *Hebeloma* spp., *Inocybe* spp., and *Russula nigricans* did not (Figs. 2.10f-h, respectively).

Rhizopogon vinicolor-type was more abundant on Douglas-fir in 5-yr-old clearcuts than all other stand types (Fig. 2.11a). Site-level relative abundance of *Rhizopogon vinicolor*-type was negatively related to richness and Shannon-Weaver diversity of the Douglas-fir ECM community ($R^2 = 0.73$ and 0.82, respectively). Adding stand age improved the R^2 of the diversity model by only 0.02. *Suillus lakei* was most abundant and frequent in 26-yr-old stands (Fig. 2.11b), but it was absent from 5-yr-old clearcut soils, and occurred in only one soil sample from 65- and 100-yr-old stands. *S. lakei* data differed among stand types.

Lactarius pubescens was the most abundant ECM on paper birch in 5-yr-old clearcuts (mean relative abundance = 27%), and was generally absent from all other sites. *Leccinum scabrum* reached its highest abundance in 5-yr-old stands (Fig. 2.12a), and was also a strong component of 26-yr-old clearcuts ($p = 0.08$ for stand type effect). Although *Thelephora terrestris* was found in low abundance on Douglas-fir seedlings, it was found only on paper birch in soil samples, and only on 5-yr-old clearcut sites, where its mean relative abundance was 6%. *Lactarius torminosus* did not differ statistically among stand types, but was absent in soil samples from 5-yr-old sites (Fig. 2.12b).

In all AIC-chosen models in which one stand age predictor variable was significant by likelihood ratio test, the other predictor variables (i.e. subzone, initiation type, and/or a transformation of stand age) were also significant, with the exception of models for two species described below. All abundance data for individual taxa in soil samples were severely overdispersed compared to the Poisson distribution, so models using the negative binomial were used. The associated dispersion parameter k, estimated by maximum likelihood, is listed for each modelled taxon on its respective figure (Figs. 2.13-2.17). Models are summarised in Table 2.5.

Model selection by AIC and AIC_C was generally unambiguous, with selection criteria values of the best models in most cases being at least 0.75 lower (better) than the second-best model. Exceptions were *Suillus lakei* and *Lactarius torminosus* (Table 2.4), for which model selection by AIC was less obvious. The best model of *S. lakei* frequency included initiation type as a predictor variable (Fig. 2.16d). The second-best model included only age variables, and its AIC_C value was only 0.34 higher than the best model. The best model was overdispersed compared to the Poisson ($chi^2/df = 2.9$), and its predictors were not significant by likelihood ratio test. The second-best model was better fit to the distribution ($chi^2/df = 1.6$), and its predictors were significant. Two models of abundance of *Lactarius torminosus* on paper birch had AIC values that were only separated by 0.2. Both models are included in Figure 2.17a.

ECM from Seedling Samples

Mean ECM colonisation of Douglas-fir was 96% and 98% on burned and clearcut 5-yr-old sites, respectively. There was a tendency for greater ECM richness, evenness, and diversity on burned than clearcut sites, but differences between stand types were not significant (Fig. 2.18a-d). Neither ordinations based on abundance nor frequency data showed significant structure in the seedling ECM communities (Monte Carlo test; $p = 0.21$ and 0.25, respectively), nor did they group sites according to initiation type. *Rhizopogon vinicolor*-type was more abundant in clearcuts, while *R. rudus* was more dominant in burned stands (Fig. 2.19).

Mean relative abundance of ECM fungal taxa on burned and clearcut 5-yr-old sites are listed in Appendix D. Four unexpected taxa were found on seedlings from 5-yr-old clearcuts: *Lactarius rubrilacteus*, Phallales 1, *Piloderma fallax* and *Russula nigricans*. There were no occurrences of *Piloderma* spp. on either host, nor were there any occurrences of any *Russula* spp. on Douglas-fir roots, in soil samples taken from the 5-yr-old sites. On the single seedling where it occurred, *Russula nigricans* colonised 66% of the root tips. *Lactarius rubrilacteus* was also found in several soil samples from older stands, and Phallales 1 from several in the 26-yr-old stands, but neither occurred in 5-yr-old clearcut soil

samples. Paper birch roots of sufficient quantity were found in soil sampled with only one Douglas-fir seedling from each of two 5-yr-old burned stands (see Appendix E for ECM on birch roots).

Discussion

Identification of ECM Fungi and Taxonomic Diversity

Rigorous morphotyping combined with DNA sequence analysis provided strong support for identification of ECM fungal taxa. The extensive use of DNA sequencing allowed unambiguous placement of most samples into unique taxonomic groupings, as well as detection of non-target fungi co-existing on root tips not necessarily recognisable by RFLPs. The main disadvantage to the sequencing approach was the common occurrence of double-peaks, likely resulting from the sensitivity of cyclosequencing reactions to non-mycorrhizal fungi co-amplified from ECM root tips (Izzo *et al.*, 2005).

Detailed morphotyping methods allowed reliable separation of taxa within cores that appeared similar under a dissecting microscope (e.g., separation of white *Piloderma* spp. variants from Phallales 1 (*Hysterangium*-like); or *Russula* spp. that do not bear cystidia from *Lactarius* spp. and other taxa, etc.). They were also useful in detecting sequence matches from non-target fungi. However, the negative relationship between molecular success and length of soil sample refrigeration suggests that a slightly less-detailed morphotyping approach would have substantially increased molecular success. Considerable time could have been saved by not examining in detail all microscopic features in difficult species groups. Rather, a more rapid morphology-based placement to genus or family would have been sufficient for most samples. Nevertheless, viewing mantle peels and/or emanating elements in appropriate stains under a compound microscope is still often necessary to achieve proper taxonomic placement and corroborate molecular results.

This study underestimated the total number of species. However, our success was similar to other studies (e.g. Cline *et al.*, 2005), and is understandable given the difficulty in adequately sampling ECM fungal communities (Taylor, 2002). The number of ECM fungal species found here is comparable to several other recent studies of above- and below-ground ECM fungi (Durall *et al.*, 2006; Horton *et al.*, 2005; Kranabetter *et al.*, 2005; Smith *et al.*, 2002; O'Dell *et al.*, 1999). Although sampling occurred only over one growing season in this study, Izzo *et al.* (2005) found ECM fungal community composition varied more between plots within one year than within plots across years. Hence, it is unlikely that ECM fungal trends identified in the current study would have been greatly different had sampling been done over additional years. No attempt was made in the current study to account for differences between spring and fall ECM fungal communities because the number of tips sampled in one season was probably grossly insufficient for accurate community representation.

The data supported our first hypothesis that ECM species richness, diversity and evenness increase with stand age. The greatest increase in richness occurred from the 5- to 26-yr-old age class, a period corresponding with tree canopy closure, and increasing only slightly thereafter, agreeing with the results of Visser *et al.* (1995) and Kranabetter *et al.* (2005). At canopy closure, tree growth rates are rapid and leaf area maximal (Simard *et al.*, 2004), with correspondingly high potential for carbon allocation to roots and mycobionts. ECM species richness and diversity tended to increase at a lower rate from 26- to 65-years, and then level off in older age classes.

The ECM community of paper birch was richer and more even than that of Douglas-fir in young stands, and increased less dramatically with stand age. Paper birch roots may remain intact and healthy following cutting or burning of shoots, providing a large carbon source and ECM legacy for stump sprouts, as well as large ECM inoculum potential for seedlings germinating nearby. By contrast, Douglas-fir does not sprout from old stumps, and seedlings are often not replanted until a few years after logging, requiring inoculation of seedlings from other plants, hyphae or spores. Jones *et al.* (1997) found that richness and evenness of the ECM fungal community on Douglas-fir was actually higher than that of paper birch at four months after outplanting, but was no different at sixteen or twenty-eight months. In that study, sites had been destumped prior to planting of both host species, supporting that birch stump sprouting could have been important in maintaining higher ECM fungal diversity on birch in the current study. Durall *et al.* (2006) found no difference in epigeous ECM sporocarp diversity among recently planted birch, Douglas-fir, and mixed stands. However, studies commonly show a strong discrepancy between above- and below-ground ECM community composition and structure (Peter *et al.*, 2001b; Durall *et al.*, 1999; Gardes & Bruns, 1996). An important cause of this discrepancy is likely the exclusion of, or difficulties in sampling, hypogeous and resupinate fruiting bodies of ECM species in sporocarp studies.

Our second hypothesis, that ECM communities differ between clearcut and burned forests, was rejected in this study. ECM community diversity was similar among 5-yr-old stands regardless of whether they originated after fire or clearcutting, and there was no grouping of 5-yr-old sites by initiation type in NMS ordinations based on their ECM fungal communities. These results suggest that fungal inoculum was not limiting on these sites, even though the fires were intense (based on forest floor observations and extent of disturbance) and would have likely reduced inoculum of many ECM fungal species (Lazaruk *et al.*, 2005; Bruns *et al.*, 2002; Grogan *et al.*, 2000; Taylor & Bruns, 1999). Although ECM richness and diversity tended to be lower in 26-yr-old burned than clearcut stands, this may have resulted from half the burned sites occurring in the ICHmk rather than the ICHmw subzone, where ECM communities were generally more diverse.

ECM Community Composition and Structure

ECM community composition varied with stand age, particularly for the Douglas-fir and combined communities, supporting our third hypothesis regarding patterns of fungal succession. Our results show that some fungal succession patterns are clearer at the genus than species taxonomic level. For example, *Russula*, one of the three most speciose genera in this study, increased in abundance and frequency with stand age. This is consistent with the other recent chronosequence studies of ECM fungal communities (Kranabetter *et al.*, 2005; Smith *et al.*, 2002; Visser, 1995). Patterns for individual *Russula* species, however, were more variable, probably because they occurred rarely. These fungi likely have patchy distributions, as suggested by the high dispersion parameter for *R. nigricans*. *R. brevipes*, *R. aeruginea*, and *R. roseipes* were similar, with infrequent occurrence but high root tip abundance where found. Patchy distributions, both horizontally as well as vertically in soil profiles, are generally expected for ECM fungi (Lilleskov E.A. *et al.*, 2004; Rosling *et al.*, 2003; Tedersoo *et al.*, 2003; Dickie *et al.*, 2002; Bidartondo *et al.*, 2000). *Russula brevipes* occurred only at the 100-yr-old sites, supporting Bergemann *et al.*'s (2002) reference to it as a "late-stage" fungus. *Russula* species were absent from young stands on Douglas-fir roots, except for extensive colonisation of a single Douglas-fir seedling by *R. nigricans*. New hosts may be infected by *Russula* species primarily from existing fungal networks, but the spread and patchy distribution may also result from within-stand spore dispersal (Redecker *et al.*, 2001). Spore germination and survival of new mycelia may, however, be sensitive to soil conditions and the presence of other soil microorganisms.

Piloderma also increased in frequency and abundance with stand age, agreeing with patterns observed by Visser (1995) and Smith *et al.* (2000). Smith *et al.* (2000) found that *Piloderma* mycelia and mycelial cord occurrence also increased with abundance of well-decayed coarse woody debris. Species of *Cortinarius* also tended to increase in frequency and abundance with stand age after 5 years. *Cortinarius* species were not found in this study as frequently as they were in ICH sporocarp studies (Durall *et al.*, 2006; Kranabetter *et al.*, 2005). Again, above- and below-ground views of ECM communities are often quite different. In this case, patchy distribution of ECM tips may have caused lower observation frequency of *Cortinarius* spp., as they were often abundant in the cores in which they were found despite their infrequence.

Rhizopogon vinicolor-type was considerably more dominant on Douglas-fir in 5-yr-old than older stands, both from soil and seedling samples. Other studies also show that *Rhizopogon* species are common following disturbance, with high frequency on seedlings grown in disturbed areas or in bioassay soils taken from wildfires or clearcuts (Grogan *et al.*, 2000; Baar *et al.*, 1999; Simard *et al.*, 1997c; Jones

et al., 1997). Douglas-fir seedlings in this study were dominated by *R. vinicolor*-type more than in other nearby studies. For example, the relative abundance of *R. vinicolor*-like on 28-month-old field-grown Douglas-fir seedlings was only 37% in Jones *et al.* (1997), roughly half of the average for the 5-yr-old stands in this study. In that study, fungi forming E-strain mycorrhizae and *Thelephora* occupied considerable portions of the ECM fungal community on Douglas-fir, but they did not in the current study. These fungi are often considered to be "early-stage" (e.g. Visser, 1995) and commonly colonise seedlings in greenhouses, but they may not compete effectively with fungi like *Rhizopogon* after a few years of growth in nature.

There are several plausible explanations for why *Rhizopogon vinicolor*-type dominated the mycorrhizal community of Douglas-fir in 5-yr-old stands. *Rhizopogon* spores are known to persist as viable ECM inocula for long periods of time, and can be abundantly and uniformly distributed even in soils where their ECM hosts are not present (Horton *et al.*, 1998), so it is not surprising that they survived where Douglas-fir roots were only patchily distributed. The rhizomorphs of *R. vinicolor* and *R. vesiculosus* may be particularly advantageous for infecting seedling roots (Simard *et al.*, 1997b), particularly after spores and inocula of many ECM fungi have declined , which usually happens within two years after logging (Hagerman *et al.*, 1999a). *R. vesiculosus* appears to spread vegetatively to several hosts more readily than *R. vinicolor* (Kretzer *et al.*, 2003a), but these species could not be analyzed separately in this study. Environmental conditions in young stands may also be well-suited to the physiology of *R. vinicolor* and *R. vesiculosus* (e.g., nutrient and moisture uptake and transfer), or young Douglas-fir may select for these host-specific fungi in this environment. Species interactions with other ECM fungi are likely important in determining community structure as well (Koide *et al.*, 2005; Jonsson *et al.*, 2001; Wu *et al.*, 1999).

Rhizopogon rudus may have been more dominant in burned than clearcut 5-yr-old stands because its spores may survive fire better than those of *R. vinicolor* and *R. vesiculosus*. Soil moisture availability may also have played a role in colonisation patterns; soils are often dry after wildfire because moderate to severe burns cause hydrophobicity of the uppermost soil layers (Certini, 2005), and this effect has been found to persist for almost two years following severe wildfire (Huffman *et al.*, 2001). Baar *et al.* (1999) found greater colonisation by *R. olivaceotintus* than *R. ochraceorubens* (= *R. occidentalis*; (Kjoller & Bruns, 2003)) in dry bioassay soil, but not in moist soil. In the present study, *R. rudus* displayed a preference for mineral soil over the forest floor (see Chapter 3), so it was likely of higher relative abundance in burned stands because forest floor was generally present in clearcut stands but absent from burned stands in the 5-yr-old age class. Timing of colonisation may also explain why different *Rhizopogon* species dominate in certain environments (Kennedy & Bruns, 2005). Cline *et al.* (2005)

found nursery grown Douglas-fir to be already heavily colonised with *R. rudus* contaminants when outplanted. The higher relative abundance of *R. rudus* on young burned than clearcut sites in the current study therefore could have simply originated from nursery stock colonisation differences.

Two birch-specific fungi, *Lactarius pubescens* and *Leccinum scabrum*, were very abundant in 5-yr-old stands, paralleling *Rhizopogon* stand age patterns on Douglas-fir. These results contrast with Mason *et al.* (1983), who refer to strand-forming *Lactarius pubescens* and *Leccinum* as "late-stage" fungi. The "late-stage" description arises from bioassay studies (Fox, 1983; Deacon & Donaldson, 1983), which demonstrated that these two fungal taxa do not readily inoculate birch from spores or mycelium dislocated from live hosts. This suggests that vegetative spread was important in young stands after limited initial colonisation by spores or fragmented mycelia, or that these fungi were legacies of pre-disturbance birch roots from which sprouts arose. It is not surprising that the dominant fungi on both hosts in 5-yr-old stands were strand-forming, as host roots are less abundant and separated by greater distances than in older stands.

These same researchers that described *Leccinum* as "late-stage" also found that *Hebeloma crustuliniforme* and *Hebeloma sacchariolens* were early colonisers, but *Hebeloma velutipes* and *H. incarnatulum* in the current study were generally absent from 5-yr-old stands and frequent in older stands. Similar to this study, Kranabetter (2005) also found that prevalence of different *Lactarius* species varied between young and mature stands. These results suggest that generalisations about ECM succession are sometimes only possible at the species level and cannot always be extrapolated to an entire genus.

Cenococcum geophilum was ubiquitous in this study, occurring at every site. Although it was not consistently dominant in root tip abundance, it was unparalleled in frequency, occurring in 51% of soil samples. Such an apparently uniform distribution may be a result of colonisation from many separate inoculum sources, as suggested by high genetic diversity of *C. geophilum* across fine spatial scales (Douhan & Rizzo, 2005). *C. geophilum* colonised birch more than Douglas-fir, which is consistent with Kernaghan *et al.*'s finding (2003) that it associated more with hardwoods than conifers in mixed boreal forests. However, Durall *et al.* (1999) found overall *C. geophilum* relative abundance on western hemlock to be near 30%, similar to its highest site-level relative abundances on birch in this study. Durall *et al.* (1999) also found *C. geophilum* relative abundance was less than 10% on lodgepole pine co-occurring with the western hemlock. While this fungus is certainly a host generalist, it may associate with some hosts more readily than others.

Network Potential between Host Species

The results of this study do not support the hypothesis that the proportion of ECM root tips colonised by shared fungi decreases with increasing stand age. In contrast, there was a lower proportion of shared ECM fungi in 5 yr-old than older stands because of the high relative abundance of a few host-specific fungi. Shared fungi nevertheless occupied a significant proportion of birch roots in young stands. Douglas-fir seedlings initially may have been dominated by shared species as suggested by Jones *et al.* (1997) and Simard *et al.* (1997c), but not when they are 4-6-years-old as in this study. While these results suggest that CMNs are likely to form in young stands, they also show that they should be considerably more extensive in older stands. The proportion of root tips colonised by shared fungi increased significantly with stand development, and was greatest on Douglas-fir in the oldest age classes. CMNs in older stands may facilitate direct transfer of carbon and nutrients from dying roots of senescent birch trees to Douglas-fir.

The few host-specific fungi dominating young stands in this study might tend to be categorized as ruderal species. However, *Rhizopogon vinicolor*-type fungi also accounted for a substantial proportion of the community in older stands, and therefore might be better categorized as competitive ("C") strategists, as described by Grime (1977) than r-selected (ruderal). *Leccinum scabrum* was also not restricted to young stands and therefore should not be thought of as a ruderal fungus. A "C"-type strategy would hold under the assumption that the soil environment in young sites following disturbance is generally less stressful than older sites. It appears that competition from other ECM fungi is likely lower in young stands, perhaps because inoculum is more limiting for other ECM species. However, soil nitrogen and phosphorus were not found to be more available in young regenerating sites than in older sites (see Chapter 3). Other unstudied ecological factors, such as microbial community structure and processes, may more strongly affect ECM community structure.

Conclusions

Stand age clearly affected diversity and structure of ECM communities in this study. Paper birch appeared important in maintaining ECM fungal diversity during stand initiation. However, its potential to form CMNs with Douglas-fir in this stand development stage was relatively low in comparison to older stands. This does not, however, preclude the indirect effects that birch and the diversity of its ECM community have on soils from playing a role in productivity and ECM colonisation of establishing Douglas-fir. Stand initiation type (i.e. wildfire or clearcut) did not appear to affect ECM diversity, community composition, or structure within the age range of stands studied. Our ability to detect differences was diminished by the short time spans represented in the two age classes for which stands of both initiation types were studied.

This study revealed some strong ECM community trends with stand development, and some were clearer at the genus level while others were apparent at the species level. Overall ECM fungal diversity increased mainly from 5- to 26-yr-old stands, but community composition and structure continued to change from 26- to 65-yr-old stands. It was not possible to draw general conclusions about relationships between fungal life-history strategies or fungal species composition with forest stand development. However, ecological traits of individual fungal species are difficult to characterise in a community study. Further autecological and population genetics studies are needed to improve our understanding of the links between plant and fungal succession in forest communities.

Figures

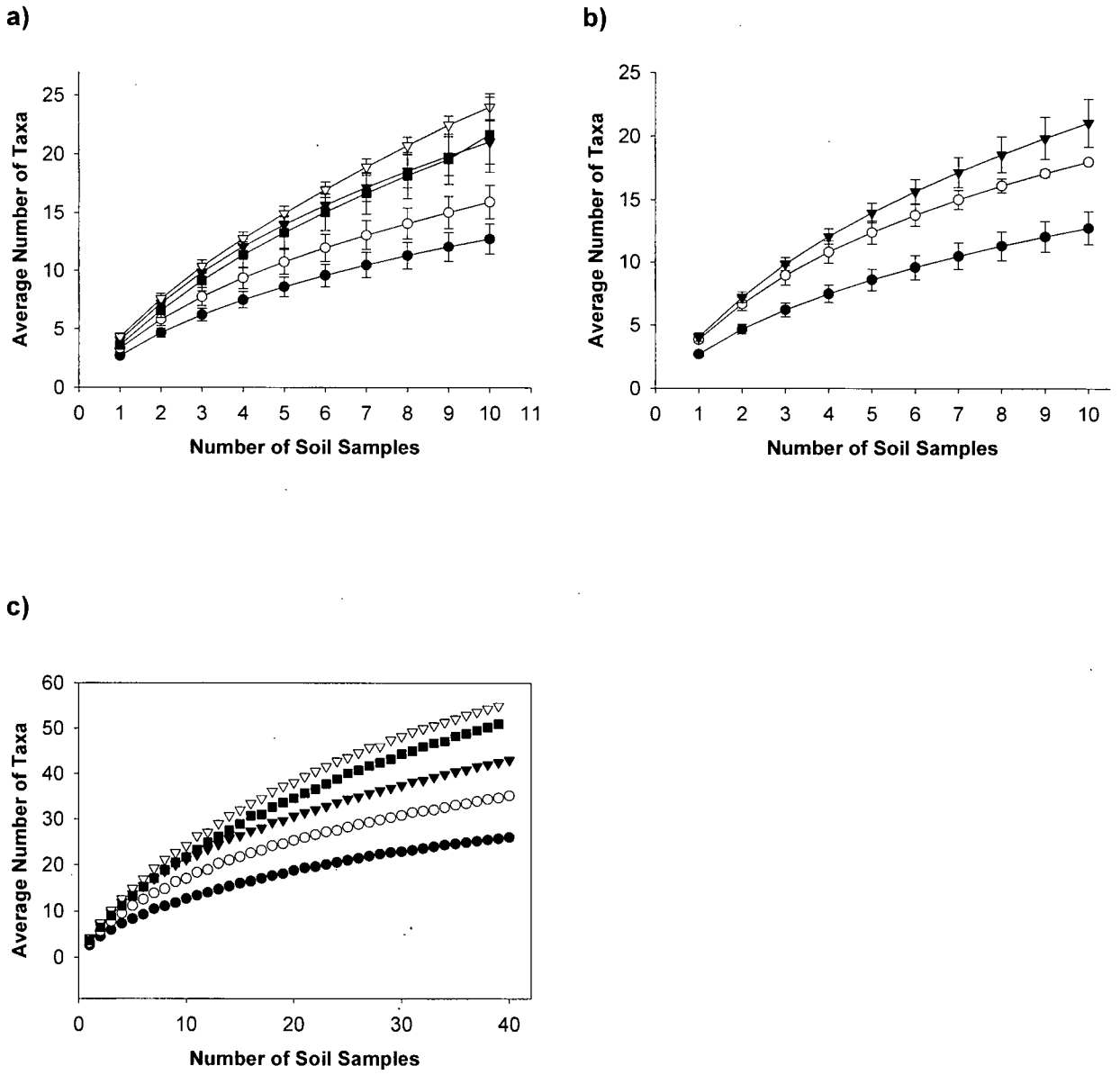


Figure 2.1 Taxa-sample unit curves for the combined community (a) site-level, including all sites; (b) site-level, excluding the 26-yr-old burned ICHmk sites and two oldest age classes, and (c) cumulative for each stand type. Filled circles = 5-yr-old clearcuts; open circles = 26-yr-old burned sites; filled triangles = 26-yr-old clearcuts; open triangles = 65-yr-old burned sites; and filled squares = 100-yr-old burned stands.

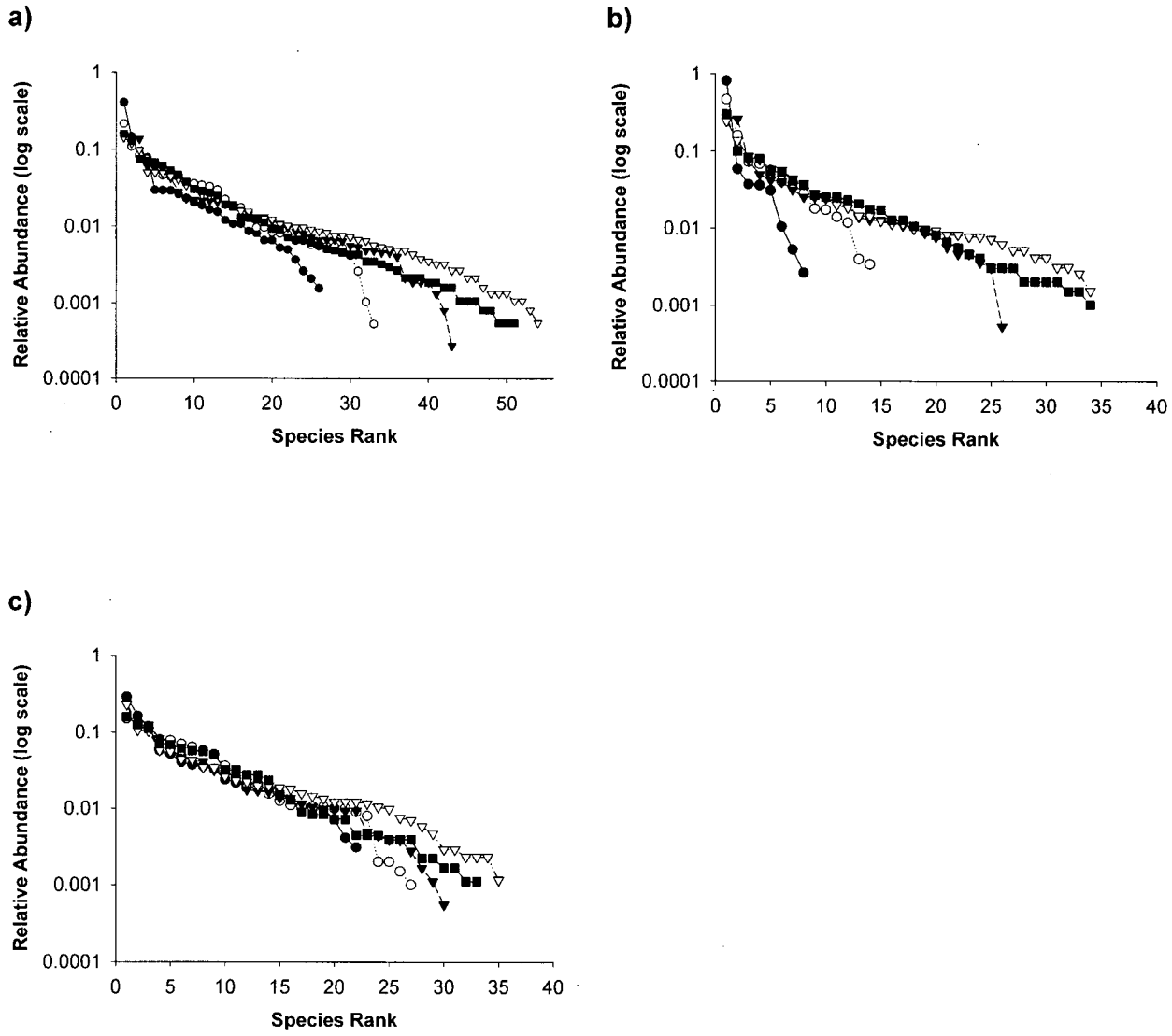


Figure 2.2 Rank abundance plots for ECM communities with (a) both hosts combined; (b) Douglas-fir; and (c) paper birch. Filled circles = 5-yr-old clearcuts; open circles = 26-yr-old burned sites; filled triangles = 26-yr-old clearcuts; open triangles = 65-yr-old burned sites; and filled squares = 100-yr-old burned stands.

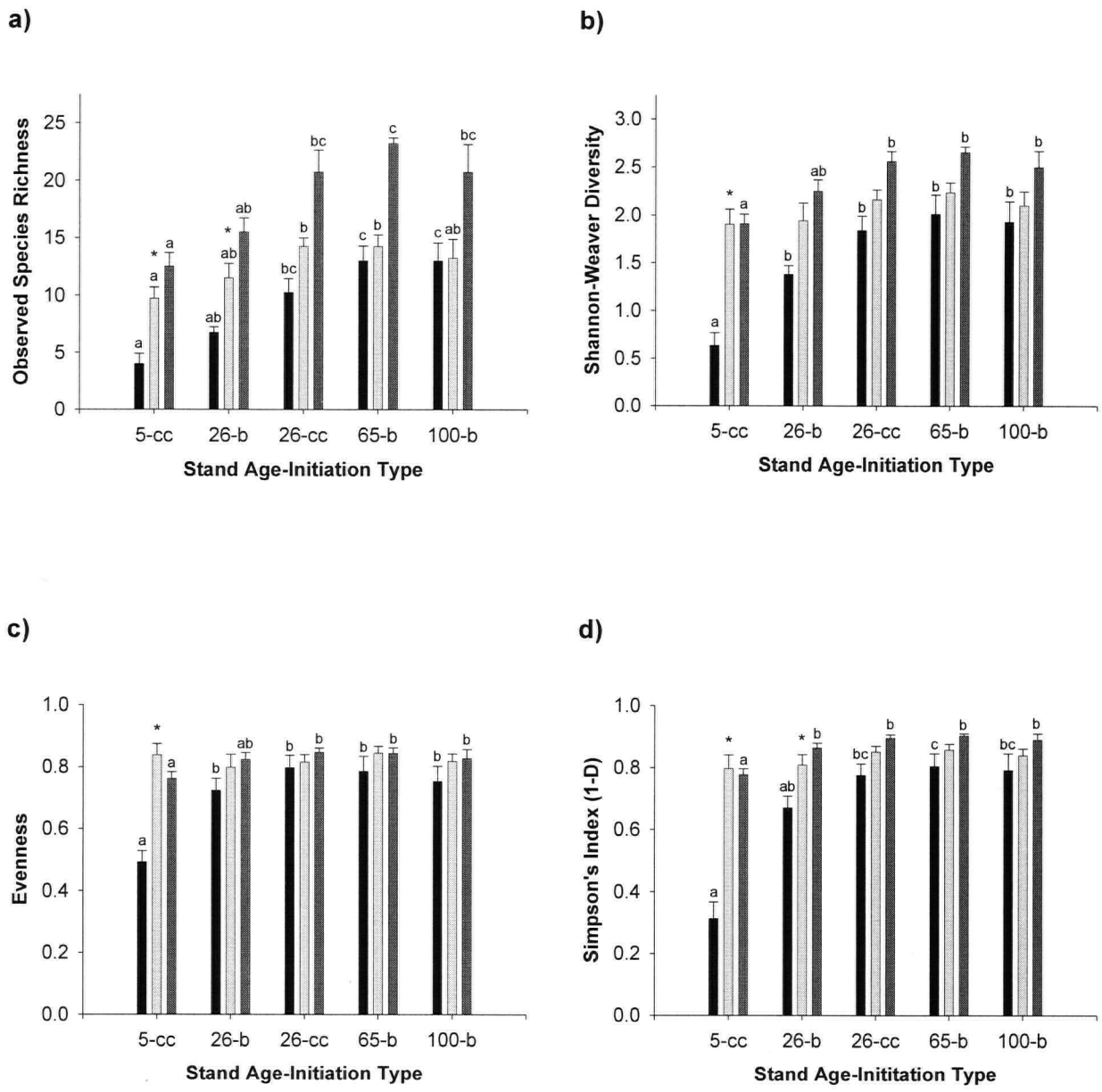


Figure 2.3 ECM community diversity variables (a-d) by stand type (cc = clearcut, b = burned); bars represent means (n = 4) and error bars represent one standard error of the mean. Black bars = Douglas-fir; light grey bars = paper birch; dark grey bars = combined community. Means within host species (i.e. with the same bar colour) that share the same letter do not differ significantly ($p > 0.05$). Bars without any letters indicate no significant difference found among stand type means for that host. * indicates a significant difference between host species within that stand type (from multiple comparisons). Significant stand type by host species interactions were detected for all analyses (split-plot ANOVA). Combined communities were analysed by separate one-way ANOVAs.

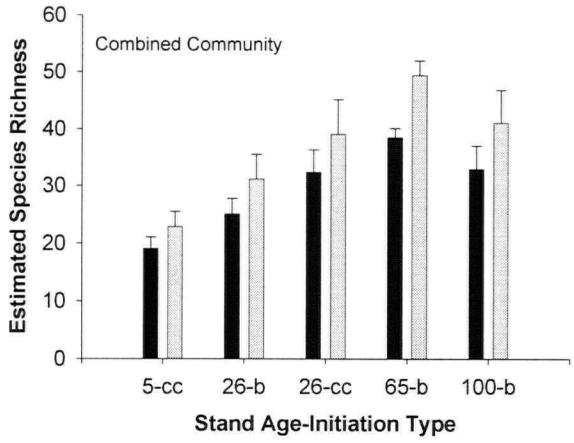


Figure 2.4 Mean (n = 4) 1st (black bars) and 2nd (grey bars) order jackknife estimates of species richness by stand type (n = 4); cc = clearcut; b = burned. Error bars represent one standard error of the mean. Not statistically tested.

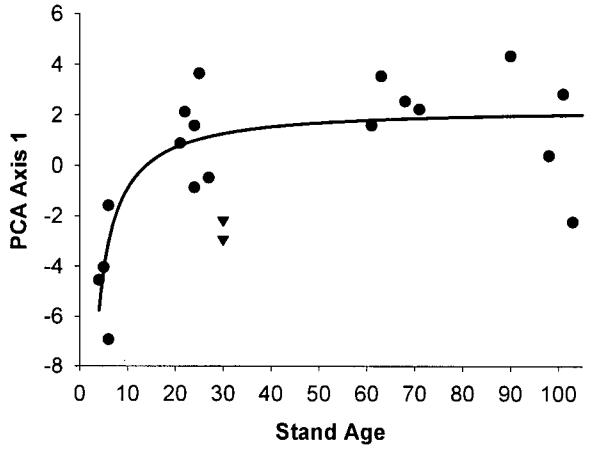


Figure 2.5 Observed site scores on first principal component axis from PCA on 13 ECM diversity variables (circles = ICHmw sites; triangles = ICHmk sites) and values predicted by the model (line) for the ICHmw subzone.

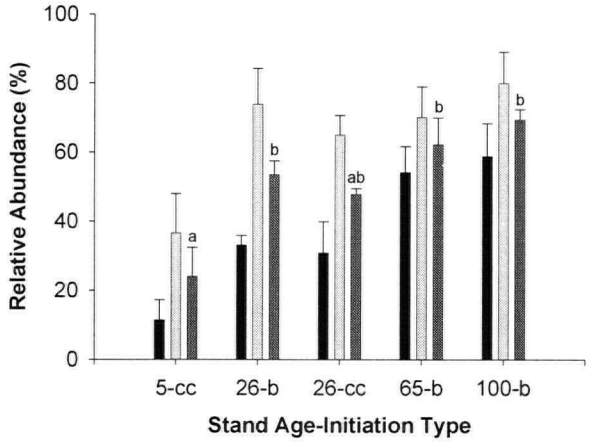


Figure 2.6 Mean percentage (n = 4) of ECM root tips colonised by fungi observed on both hosts by stand type. Black bars = Douglas-fir community; light grey bars = paper birch community; dark grey bars = combined community (expressed as average values of Douglas-fir and paper birch communities); cc = clearcut; b = burned. Combined community means with the same letters are not significantly different ($p > 0.05$). No significant stand type by host species interaction found (split-plot ANOVA). Host species effect was significant ($p < 0.05$), but multiple comparisons between hosts within each stand type detected no significant differences.

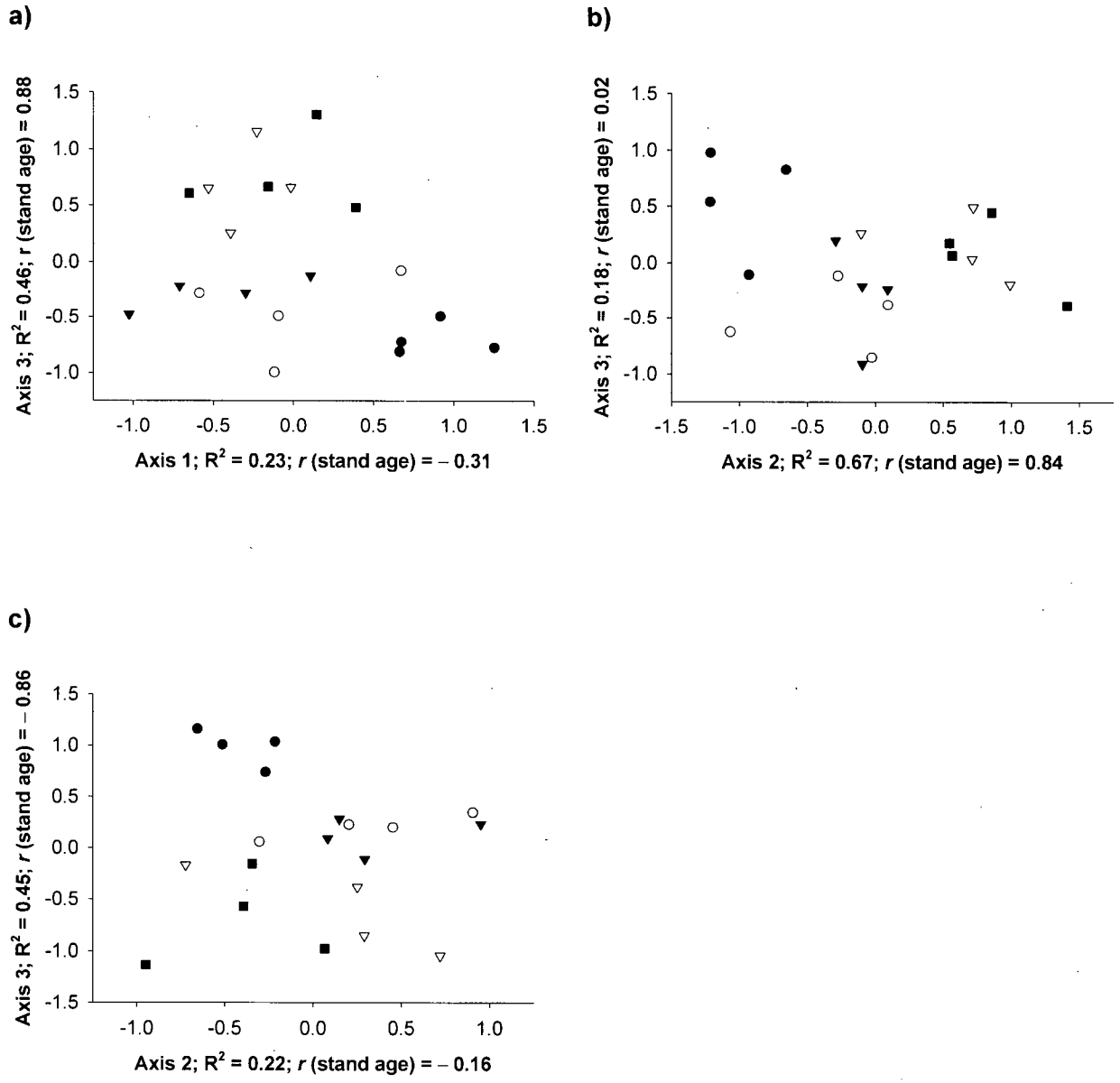


Figure 2.7 NMS ordinations of sites based on the combined ECM fungal community of both hosts using (a) species frequency; (b) frequency of species lumped into genera; and (c) species abundance. Filled circles = 5-yr-old clearcut; open circles = 26-yr-old burned sites; filled triangles = 26-yr-old clearcut sites; open triangles = 65-yr-old burned sites; and squares = 100-yr-old burned sites. R^2 values represent the proportion of total variation in Relative Sorensen distance among sites explained by ordination axes. Correlations of stand age to ordination axes are Pearson's *r*.

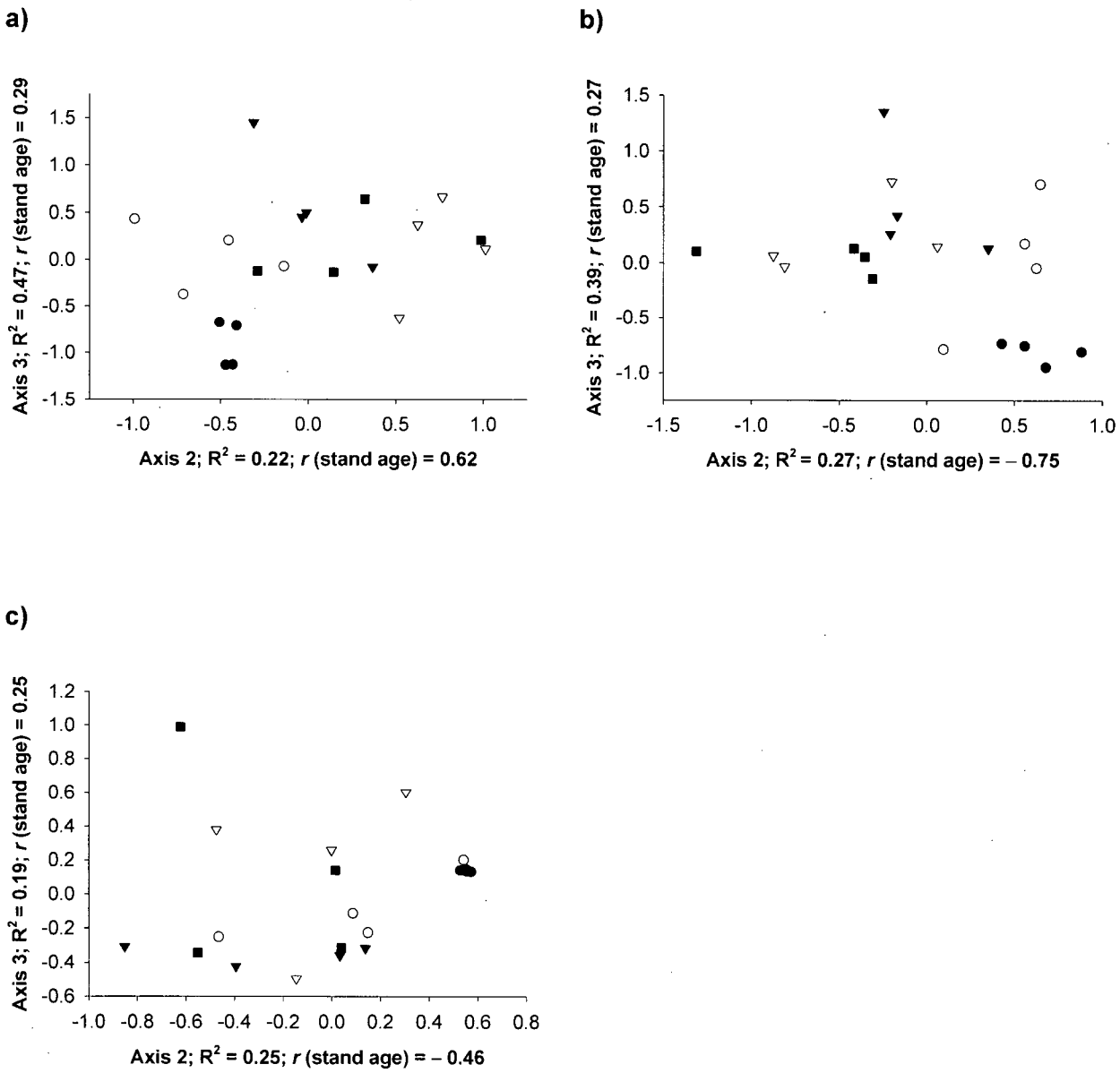


Figure 2.8 NMS ordinations of sites based on ECM fungal communities on Douglas-fir using (a) species frequencies; (b) frequency of species lumped into genera; and (c) by species abundance. R^2 values represent the proportion of total variation in Relative Sorensen distance among sites explained by ordination axes. Correlations of stand age to ordination axes are Pearson's *r*. Symbols are as in Figure 2-7.

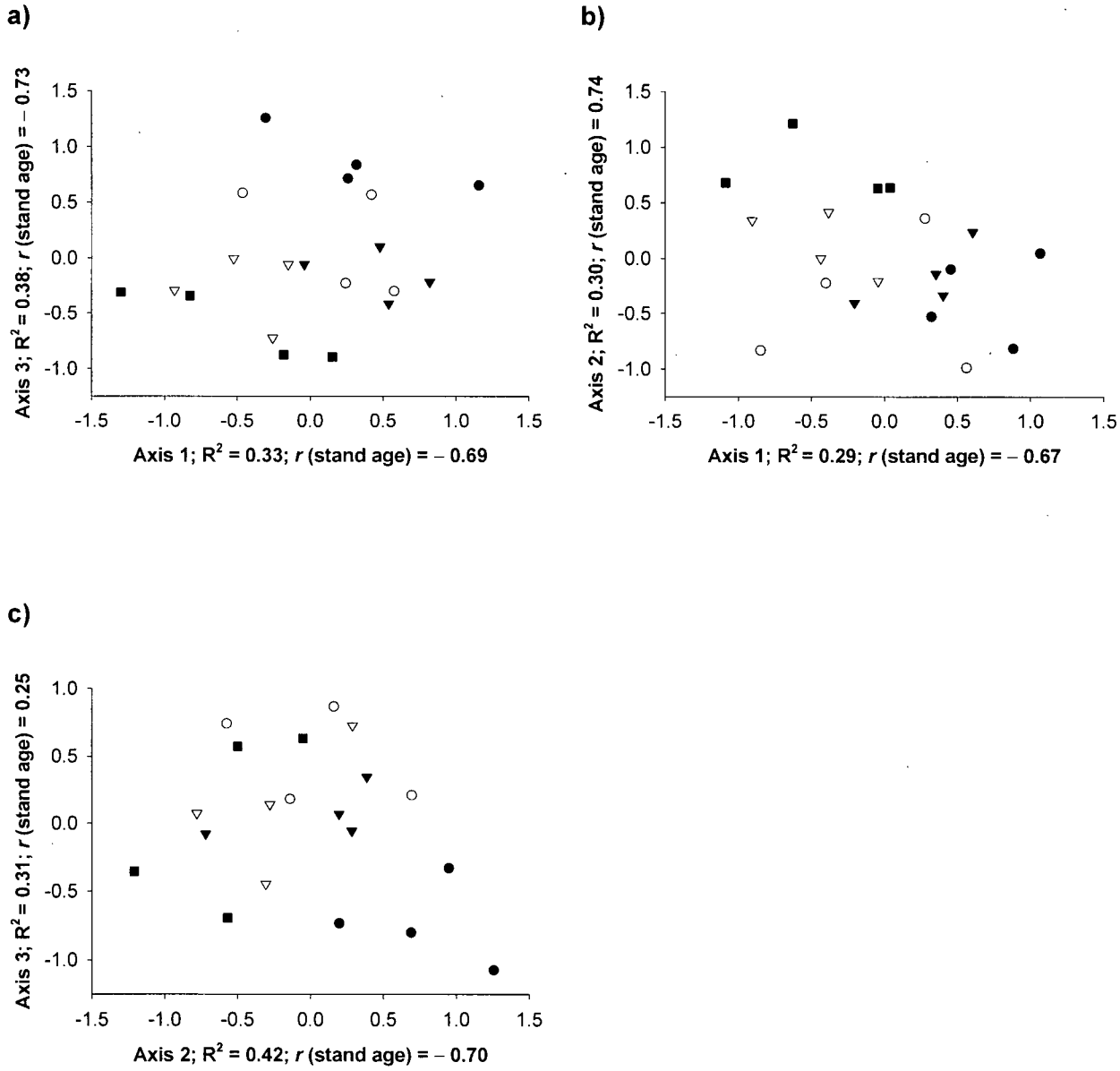
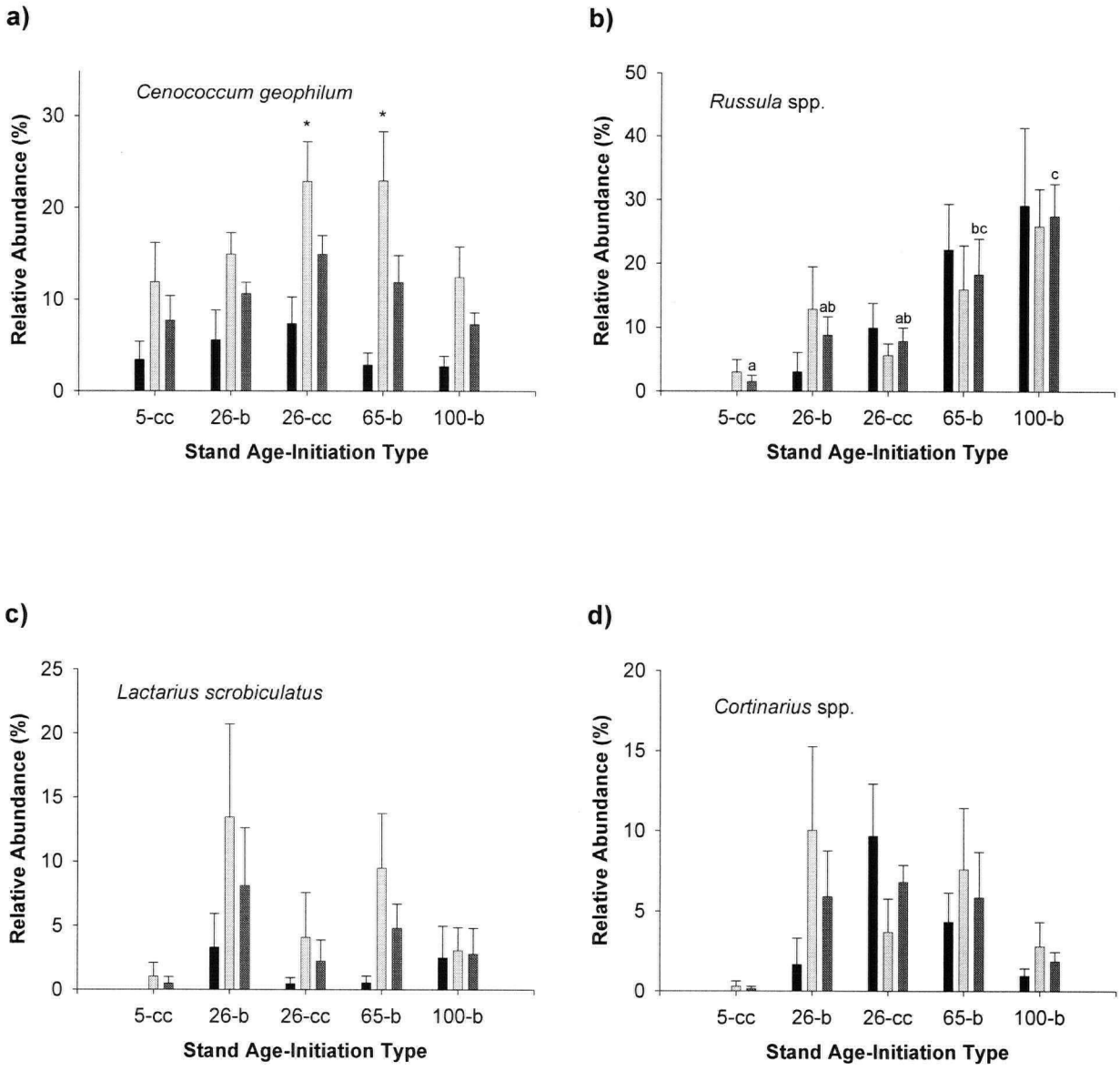


Figure 2.9 NMS ordinations of sites based on ECM fungal communities on paper birch using (a) species frequencies; (b) frequency of species lumped into genera; and (c) by species abundance. R^2 values represent the proportion of total variation in Relative Sorensen distance among sites explained by ordination axes. Correlations of stand age to ordination axes are Pearson's *r*. Symbols are as in Figure 2-7.


a)
Cenococcum geophilum
Relative Abundance (%)
0
10
20
30
*
*
5-cc
26-b
26-cc
65-b
100-b
Stand Age-Initiation Type
b)
Russula spp.
Relative Abundance (%)
0
10
20
30
40
50
a
ab
ab
bc
c
5-cc
26-b
26-cc
65-b
100-b
Stand Age-Initiation Type
c)
Lactarius scrobiculatus
Relative Abundance (%)
0
5
10
15
20
25
5-cc
26-b
26-cc
65-b
100-b
Stand Age-Initiation Type
d)
Cortinarius spp.
Relative Abundance (%)
0
5
10
15
20
5-cc
26-b
26-cc
65-b
100-b
Stand Age-Initiation Type

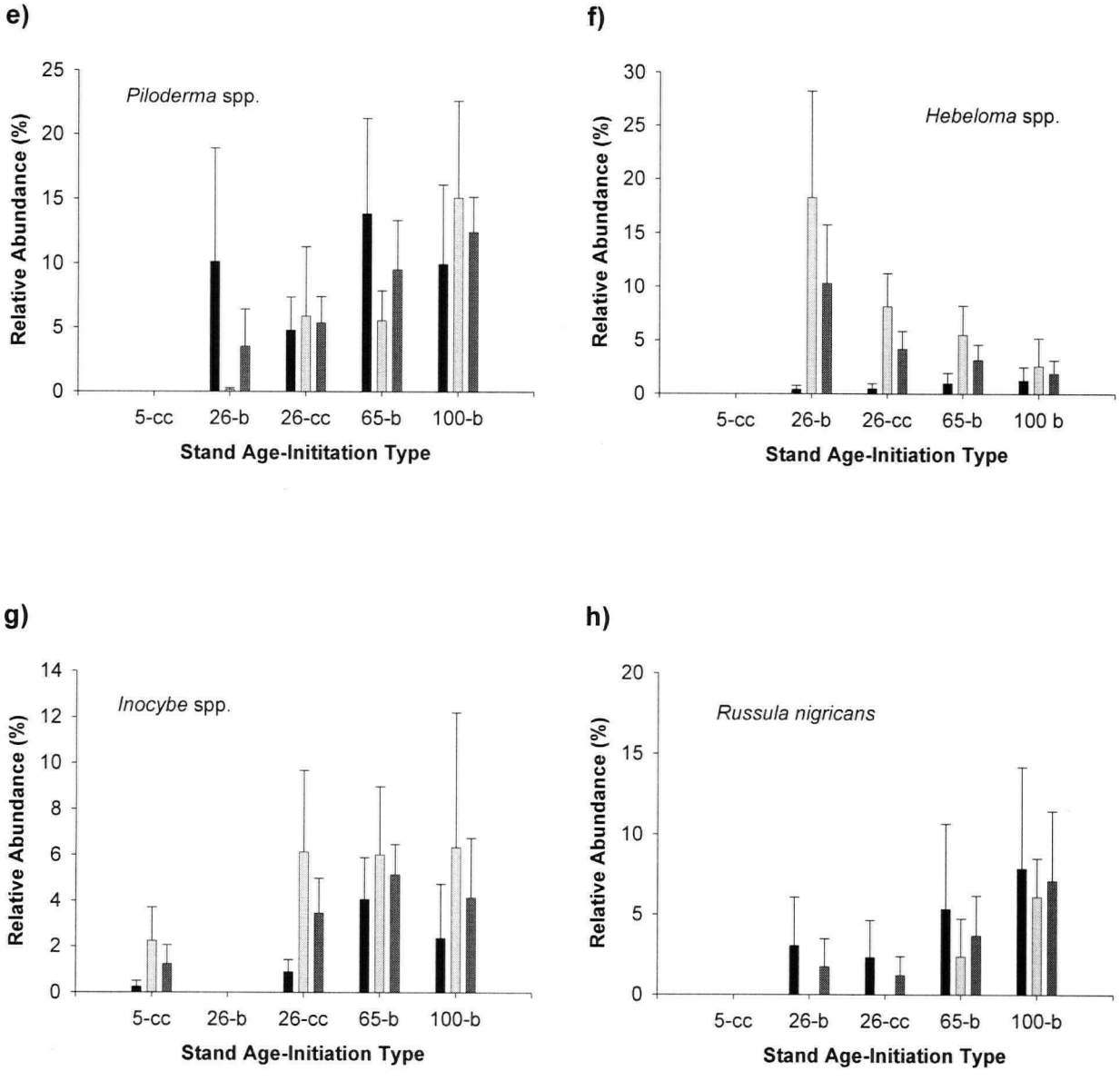


Figure 2.10 Mean relative abundances (n = 4) of fungal taxa that occurred on both hosts by stand type (a-h; cc = clearcut, b = burned). Black bars = Douglas-fir; light grey bars = paper birch; and dark grey bars = combined community. Error bars represent one standard error of the mean. Means within host species (i.e. with the same bar colour) that share the same letter do not differ significantly ($p > 0.05$). * indicates a significant difference between host species within that stand age-initiation type ($p < 0.05$). Split-plot ANOVA used for a-c; no stand type by host species interactions found; (a) no stand type effect; (b) no host species effect; c) no stand type effect; host species effect significant ($p < 0.05$), but not in mean comparisons. Kruskal-Wallis test used for d-h; (d) and (e) showed significant stand type effect; (f)-(h) showed no significant stand type effect.

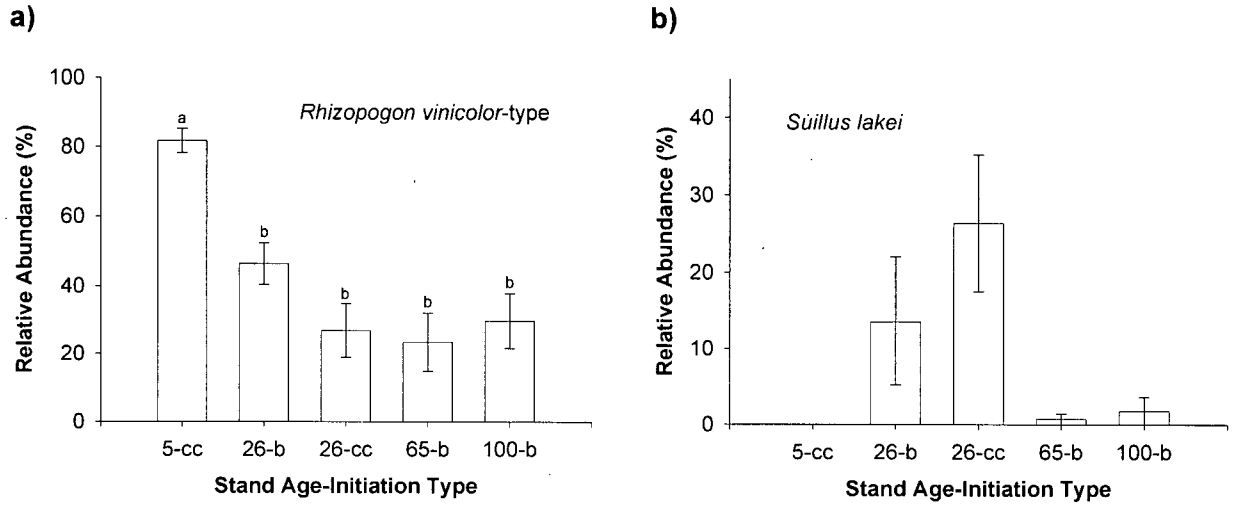


Figure 2.11 Mean relative abundances (n = 4) of two host-specific fungi on Douglas-fir by stand age-initiation type (n = 4); cc = clearcut, b = burned. Error bars represent one standard error of the mean. Means with the same letter are not significantly different ($p > 0.05$). *R. vinicolor*-type tested by one-way ANOVA; *S. lakei* showed significant stand type effect by Kruskal-Wallis test ($p < 0.05$).

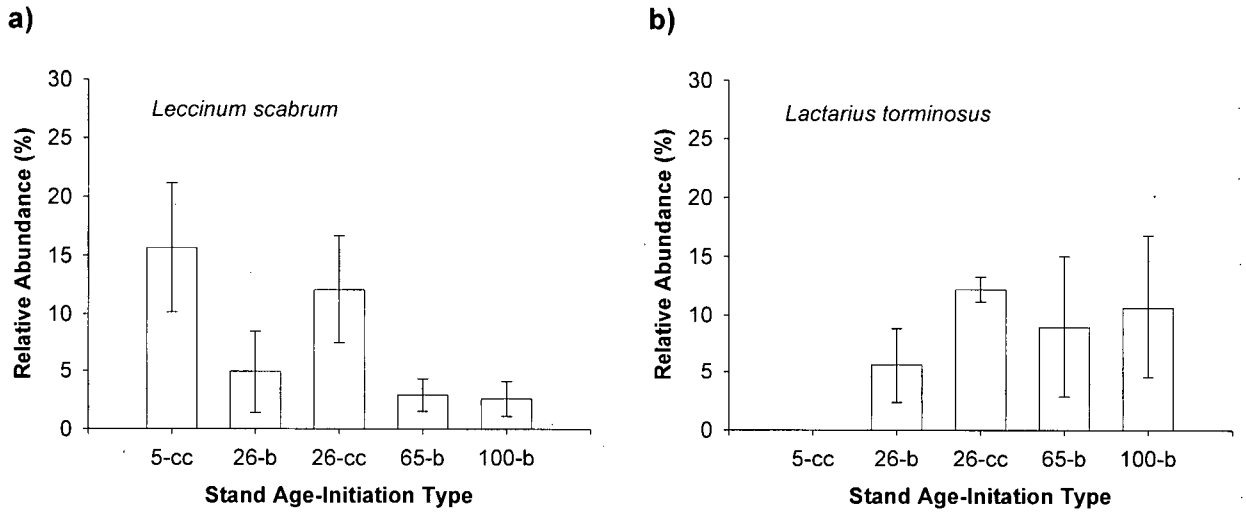


Figure 2.12 Mean relative abundances (n = 4) of two host specific fungi on paper birch by stand type. Error bars represent one standard error of the mean. No significant stand type effect for either species (one-way ANOVA; $p > 0.05$).

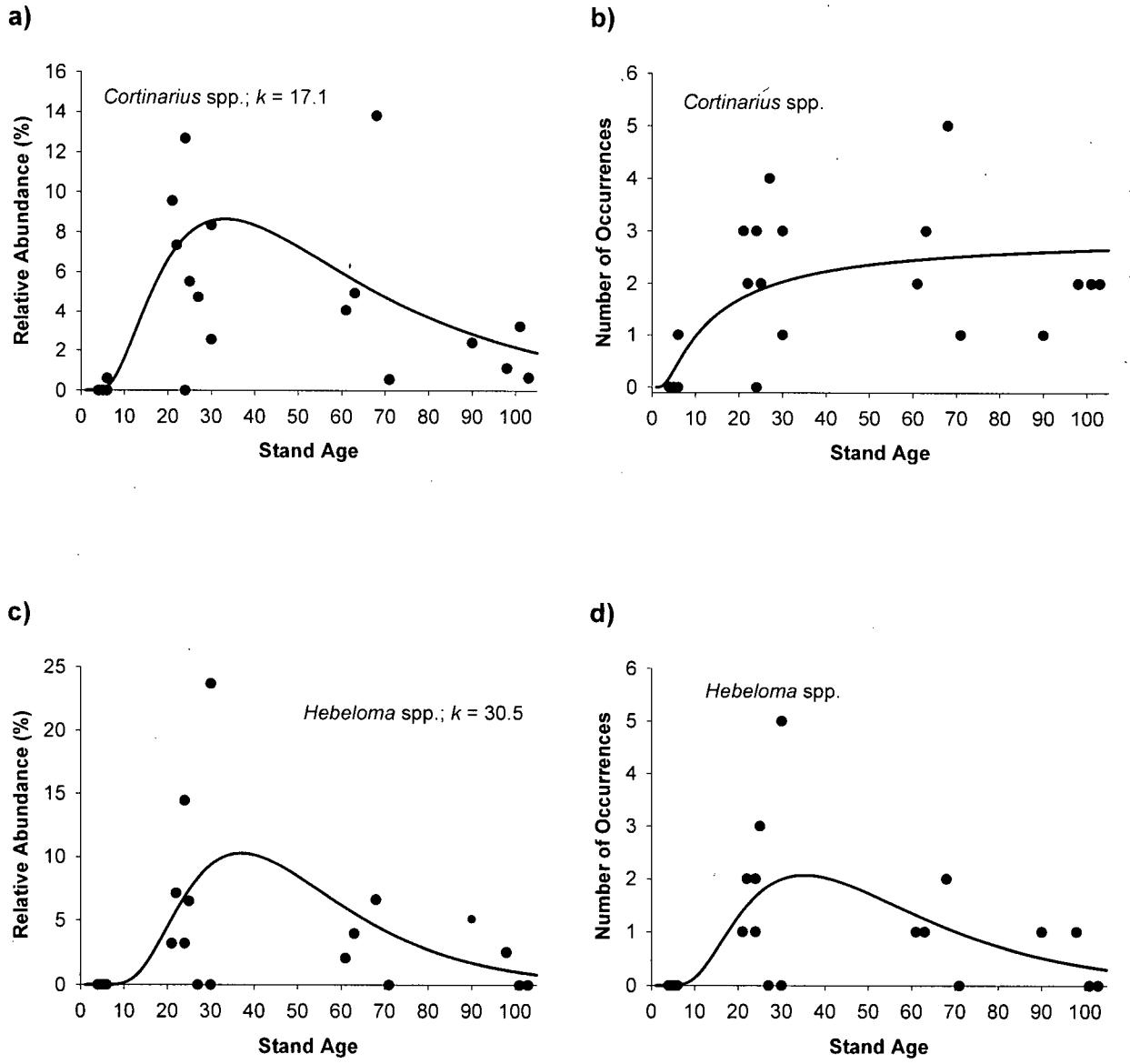


Figure 2.13 Maximum likelihood models (curves) for *Cortinarius* and *Hebeloma* spp. in the combined community. Predicted relative abundances were estimated by dividing predictions of per soil sample taxon abundance by the average number of ECM root tips per soil sample. Points are site-level values of relative abundance and number of occurrences (frequency).

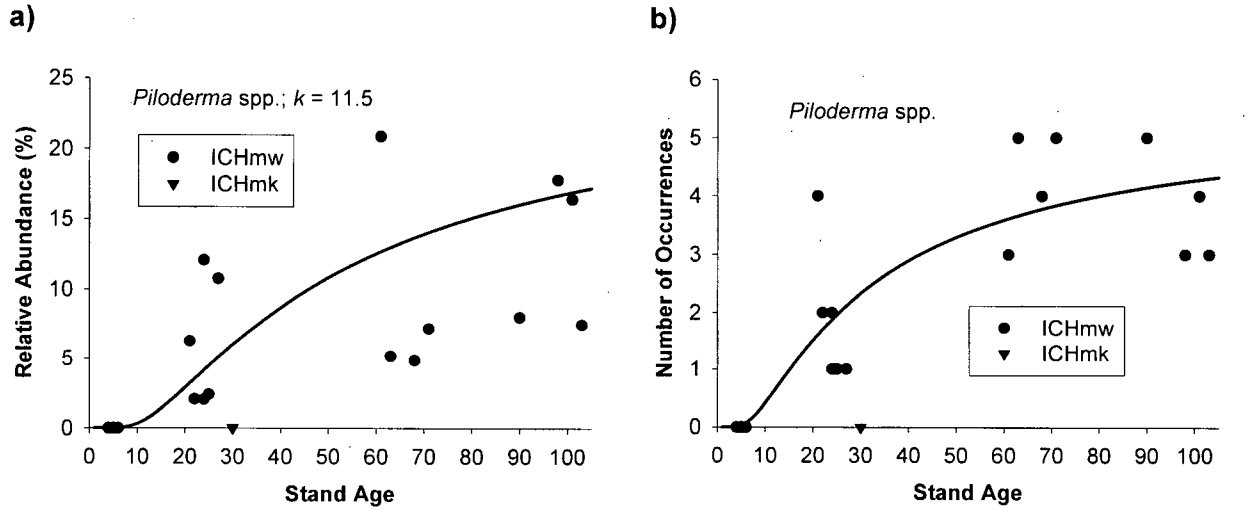


Figure 2.14 Maximum likelihood models (curves show predictions for ICHmw only) for *Piloderma* spp. in the combined community (a-b). Predicted relative abundances were estimated by dividing predictions of per soil sample taxon abundance by the average number of total ECM root tips per soil sample. Points are site-level values of relative abundance and number of occurrences (frequency).

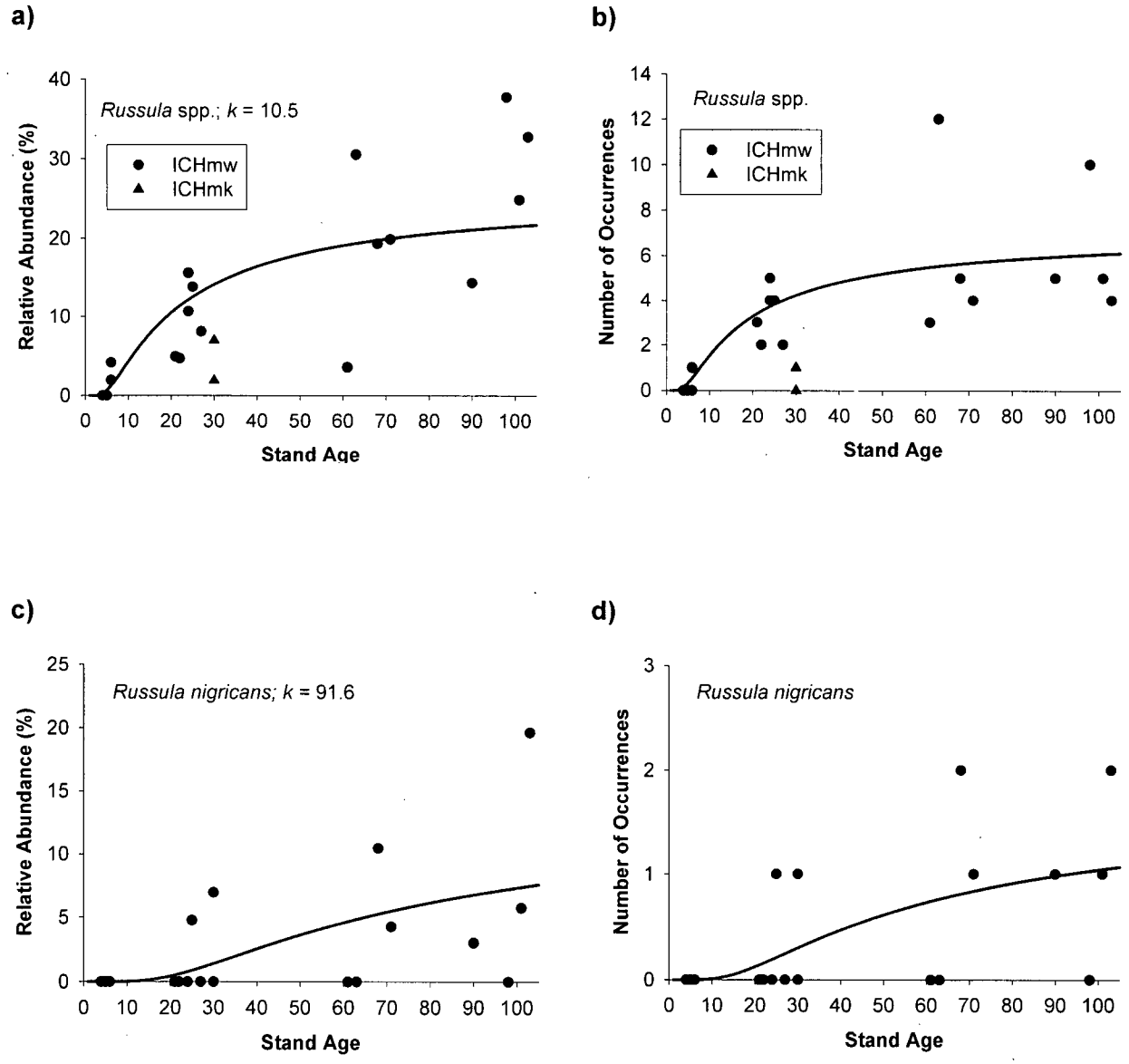


Figure 2.15 Maximum likelihood models (curves) for *Russula* spp. and *R. nigricans* in the combined community (lines show predictions for ICHmw only in (a) and (b)). Predicted relative abundances were estimated by dividing predictions of per soil sample taxon abundance by the average number of total ECM root tips per soil sample. Points are site-level values of relative abundance and number of occurrences (frequency).

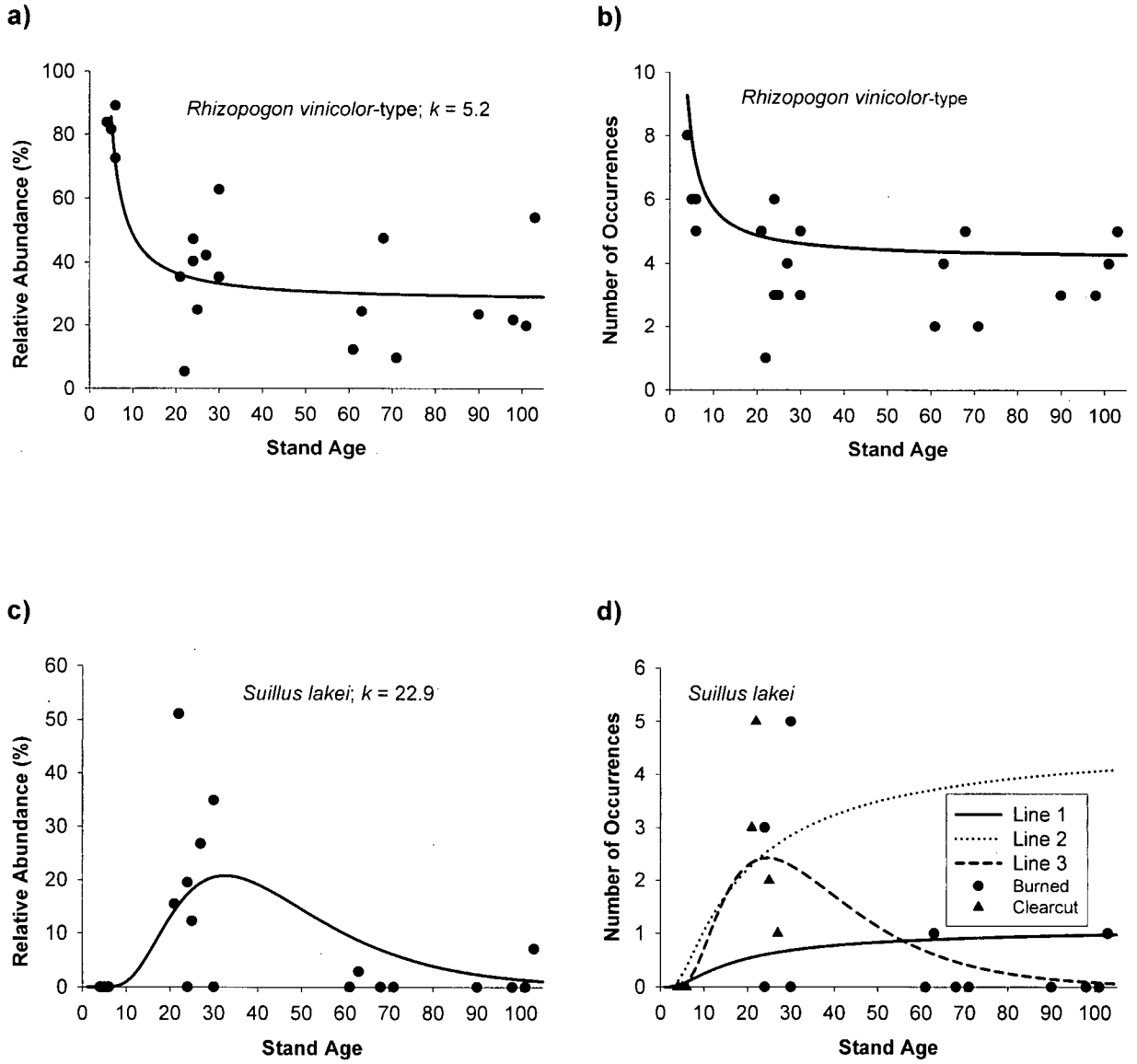


Figure 2.16 Maximum likelihood models (curves) for two host specific ECM species on Douglas-fir. Predicted relative abundances were estimated by dividing predictions of per soil sample taxon abundance by the average number of Douglas-fir ECM root tips per soil sample. Points are site-level values of relative abundance and number of occurrences (frequency). In (d), Lines 1 and 2 represent predictions for burned and clearcut sites, respectively, from the best model. Line 3 represents the second-best model, in which stand initiation type was not a predictor.

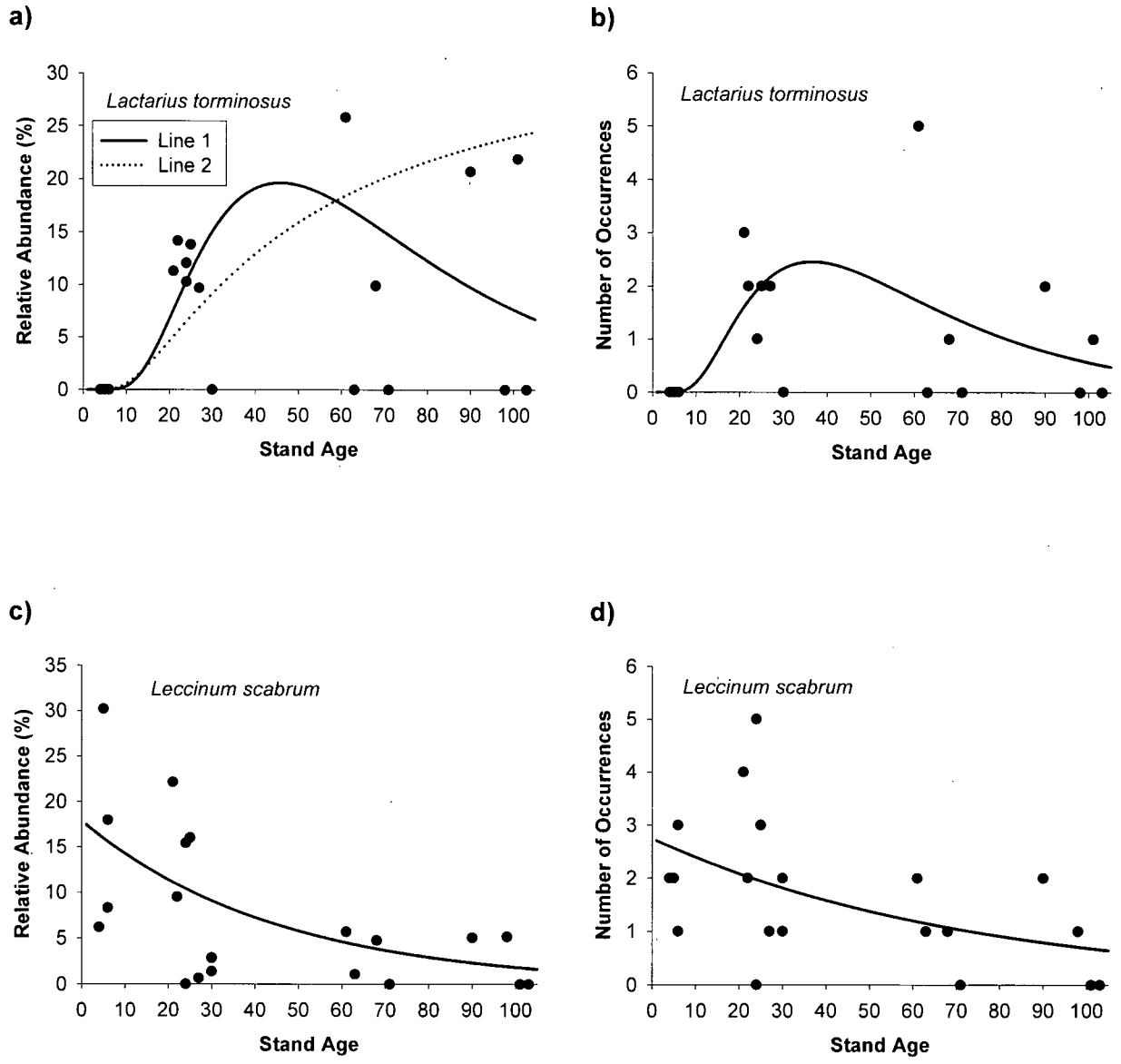


Figure 2.17 Maximum likelihood models (curves) for two host specific ECM species on paper birch. Predicted relative abundances were estimated by dividing predictions of per soil sample taxon abundance by the average number of birch ECM root tips per soil sample. Points are site-level values of relative abundance and number of occurrences (frequency). In (a), Lines 1 and 2 represent the best and second-best models, respectively.

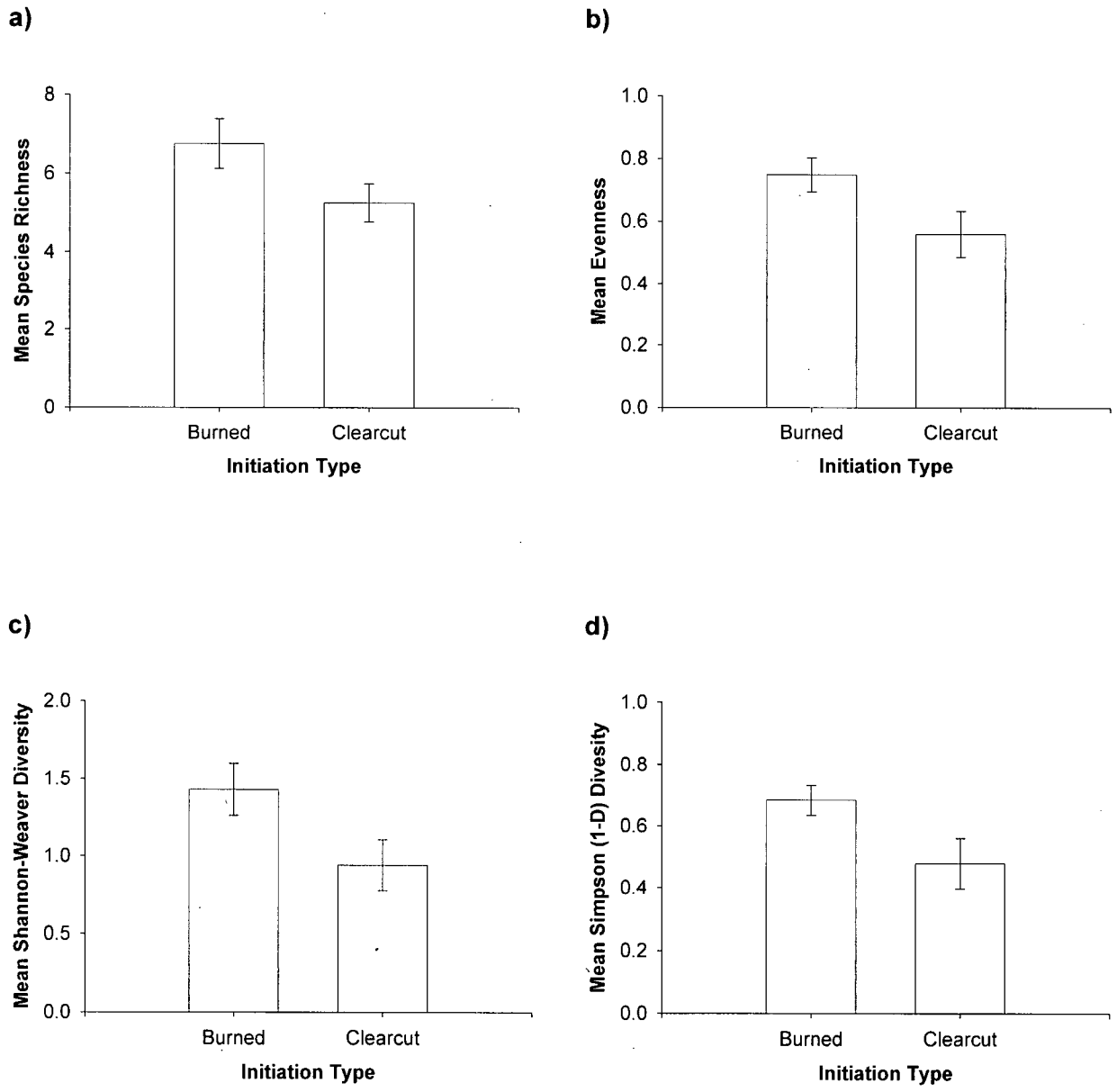


Figure 2.18 Comparison of mean ECM community diversity measures (n = 4) for Douglas-fir seedlings between stand initiation types in 5-yr-old stands. Error bars represent one standard error of the mean. Tested by two-sample t-test (two-tailed): (a) t = -1.9, p = 0.11; (b) t = -2.1, p = 0.08; (c) t = -2.1, p = 0.08; (d) t = -2.1, p = 0.08.

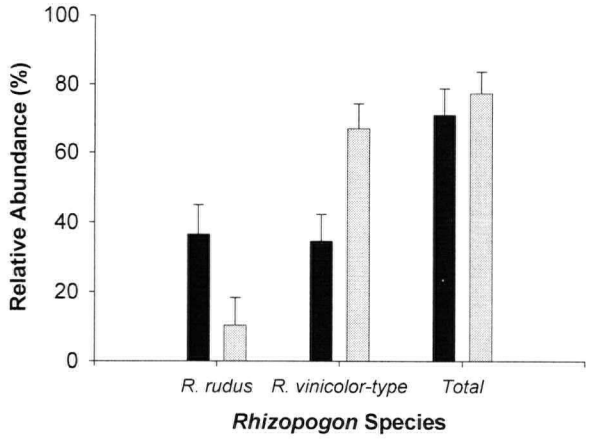


Figure 2.19 Comparison of mean relative abundance (n = 4) of *Rhizopogon rudus* and *R. vinicolor*-type on Douglas-fir seedlings between 5-yr-old burned and clearcut stands. Black bars = wildfire origin; grey bars = clearcut origin. Error bars represent one standard error of the mean. Not statistically tested.

Tables

Table 2.1 Site locations and characteristics. Interior Cedar-Hemlock biogeoclimatic subzones are defined in Lloyd *et al.* (1990).

Site	Age	Initiation Type	ICH Subzone	Elevation (meters)	Latitude/ Longitude	Crown Closure	Soil Texture[1]	Tree Species Composition[2]		
								Douglas-fir	Paper birch	Other ECM broadleaf spp.
19MR	6	clearcut	mw3	700	N 50° 58' 42" W 118° 35' 40"	50%	SiL	10%	65%	2.5%
AL	6	clearcut	mw2	600	N 50° 32' 43" W 118° 52' 49"	45%	SL	25%	50%	7.5%
BC	4	clearcut	mw2	750	N 50° 38' 48" W 118° 45' 36"	65%	SiL	5%	85%	5%
WL	5	clearcut	mw3	700	N 50° 53' 51" W 119° 16' 27"	50%	fSL	10%	70%	2.5%
IDA1	5	wildfire	mw2	1100	N 50° 38' 54" W 119° 18' 18"	30%	SCL	15%	60%	10%
IDA2	4	wildfire	mw2	950	N 50° 39' 29" W 119° 18' 03"	25%	SL	10%	65%	5%
IDA3	5	wildfire	mw2	650	N 50° 40' 00" W 119° 18' 57"	35%	SL	7.5%	80%	2.5%
IDA4	5	wildfire	mw2	500	N 50° 39' 42" W 119° 19' 57"	30%	SL	5%	80%	2.5%
ED1	30	wildfire	mk2	1200	N 50° 44' 19" W 119° 23' 09"	80%	SiL	35%	40%	12.5%
ED2	30	wildfire	mk2	1000	N 50° 44' 12" W 119° 22' 17"	85%	SiL	35%	45%	5%
MA1	24	wildfire	mw3	930	N 50° 55' 20" W 118° 50' 41"	70%	SL	40%	45%	10%
MA2	24	wildfire	mw3	975	N 50° 55' 3" W 118° 50' 56"	70%	SL	45%	40%	7.5%

Site	Age	Initiation Type	ICH Subzone	Elevation (meters)	Latitude/ Longitude	Crown Closure	Soil Texture[1]	Tree Species Composition[2]		
								Douglas-fir	Paper birch	Other ECM broadleaf spp.
DISC	27	clearcut	mw2	600	N 50° 32' 31" W 118° 52' 33"	65%	SL	40%	60%	0%
NM	21	clearcut	mw2	550	N 50° 36' 15" W 118° 40' 47"	60%	SL	40%	45%	5%
SRC	22	clearcut	mw2	900	N 50° 43' 04" W 119° 06' 54"	75%	vfSL	47.5%	50%	2.5%
ZP	25	clearcut	mw2	650	N 50° 36' 34" W 118° 39' 47"	70%	SL	40%	40%	10%
BA	63	wildfire	mw2	700	N 50° 34' 03" W 118° 50' 50"	85%	SL	45%	50%	0%
MARA	71	wildfire	mw2	600	N 50° 39' 28" W 119° 03' 49"	80%	SL	45%	50%	0%
RR	61	wildfire	mw2	800	N 50° 41' 55" W 118° 46' 07"	75%	SL	40%	60%	0%
SL	68	wildfire	mw2	700	N 50° 22' 07" W 118° 32' 03"	90%	SL	45%	45%	2.5%
4WD	103	wildfire	mw2	550	N 50° 36' 47" W 118° 50" 26"	90%	SL	50%	45%	0%
ACR	98	wildfire	mw2	600	N 50° 37' 25" W 118° 46' 06"	80%	LS	55%	30%	0%
BBP	101	wildfire	mw2	750	N 50° 27' 17" W 118° 49' 30"	80%	SL	40%	40%	0%
WAP	90	wildfire	mw2	650	N 50° 45' 1" W 118° 34' 12"	80%	SL	50%	40%	0%

[1] SiL = silty loam; SL = sandy loam; SCL = sandy clay loam; LS = loamy sand; f = fine; vf =very fine.
[2] Tree species compositional percentages were estimated as each species' or group's proportion of the total estimated canopy cover.

Table 2.2 Analysis of Variance table for split-plot analyses. n = 4 replicates.

Variation Source	Degrees of Freedom	Critical F-value (alpha = 0.05); F-test Denominator
Stand-type (age/initiation type)	4	3.06; Error 1
Error 1	15	
Host Species	1	4.54; Error 2
Stand-type * Host species	4	3.06; Error 2
Error 2	15	
Total	39	

Table 2.3 Statistical analysis table for differences among stand type means of ECM fungal diversity variables and relative abundances. Fd = Douglas-fir; Ep = paper birch. SP = split-plot; K-W = Kruskal-Wallis. See associated figures for further details.

Variable	Host	Statistical Test	F-ratio or χ^2 value: effect of stand type	P-value	F-ratio: effect of host species	P-value	F-ratio: stand type x host sp. interaction	P-value	Associated figure
Species richness	Both	SP ANOVA	8.45	0.0009	36.14	<0.0001	3.89	0.0231	2.3a
Shannon diversity	Both	SP ANOVA	7.67	0.0014	44.65	<0.0001	6.86	0.0024	2.3b
Evenness	Both	SP ANOVA	4.68	0.0118	25.69	0.0001	7.00	0.0022	2.3c
Simpson diversity	Both	SP ANOVA	19.2	<0.0001	162.06	<0.0001	20.47	<0.0001	2.3d
Relative Abundances									
Shared ECM species	Both	SP ANOVA	9.49	0.0005	23.34	0.0002	0.63	0.6454	2.6
Cenococcum geophilum	Both	SP ANOVA	2.02	0.1438	37.18	<0.0001	1.17	0.3635	2.10a
Russula spp.	Both	SP ANOVA	7.25	0.0019	<0.01	0.9638	0.52	0.7203	2.10b
Lactarius scrobiculatus	Both	SP ANOVA	1.28	0.3200	9.85	0.0068	1.64	0.2162	2.10c
Cortinarius spp.	Both	K-W	9.96	0.0410	N/A	N/A	N/A	N/A	2.10d
Piloderma spp.	Both	K-W	11.99	0.0174	N/A	N/A	N/A	N/A	2.10e
Hebeloma spp.	Both	K-W	4.94	0.2939	N/A	N/A	N/A	N/A	2.10f
Inocybe spp.	Both	K-W	6.37	0.1508	N/A	N/A	N/A	N/A	2.10g
Russula nigricans	Both	K-W	5.13	0.2379	N/A	N/A	N/A	N/A	2.10h
Rhizopogon vinicolor-type	Fd	One-way ANOVA	11.51	0.0002	N/A	N/A	N/A	N/A	2.11a
Suillus lakei	Fd	K-W	10.89	0.0279	N/A	N/A	N/A	N/A	2.11b
Leccinum scabrum	Ep	One-way ANOVA	2.58	0.0804	N/A	N/A	N/A	N/A	2.12a
Lactarius torminosus	Ep	One-way ANOVA	1.36	0.2946	N/A	N/A	N/A	N/A	2.12b

Table 2.4 Mean diversity measurements calculated from frequency data for Douglas-fir, paper birch, and combined ECM communities. Numbers in parentheses are one standard error of the mean.

Host species; Stand-Type	Mean Evenness	Mean Shannon-Weaver Diversity	Mean Simpson Diversity (1-D)
Douglas-fir			
5-yr-old clearcut	0.756 (0.058)	1.022 (0.25)	0.529 (0.106)
26-yr-old burned	0.903 (0.023)	1.719 (0.07)	0.788 (0.016)
26-yr-old clearcut	0.935 (0.012)	2.155 (0.12)	0.862 (0.015)
65-yr-old burned	0.932 (0.007)	2.376 (0.11)	0.886 (0.015)
100-yr-old burned	0.935 (0.014)	2.379 (0.15)	0.880 (0.023)
Paper birch			
5-yr-old clearcut	0.920 (0.021)	2.054 (0.17)	0.841 (0.035)
26-yr-old burned	0.890 (0.010)	2.156 (0.12)	0.846 (0.015)
26-yr-old clearcut	0.930 (0.009)	2.450 (0.08)	0.896 (0.008)
65-yr-old burned	0.901 (0.019)	2.386 (0.10)	0.871 (0.015)
100-yr-old burned	0.955 (0.009)	2.446 (0.14)	0.897 (0.013)
Both Hosts			
5-yr-old clearcut	0.898 (0.016)	2.233 (0.15)	0.862 (0.023)
26-yr-old burned	0.906 (0.011)	2.459 (0.08)	0.892 (0.009)
26-yr-old clearcut	0.941 (0.006)	2.844 (0.10)	0.929 (0.007)
65-yr-old burned	0.923 (0.007)	2.920 (0.04)	0.929 (0.004)
100-yr-old burned	0.939 (0.008)	2.829 (0.11)	0.927 (0.008)

Table 2.5 Summary of generalized linear models, estimated by maximum likelihood, of taxon abundances and frequencies. Abundance models are based on per core abundances and the negative binomial distribution. Frequency models are based on occurrences per site and the Poisson distribution. SA = stand age; SBZ = biogeoclimatic subzone (ICHmw or ICHmk). "ICHmk effect" column details the effect of this subzone in comparison to the ICHmw subzone. Significant variables gave $p < 0.05$ for likelihood ratio tests.

	Abundance Model				Frequency Model			
	Variables included	Significant variables	ICHmk effect	Fig.	Variables included	Significant variables	ICHmk effect	Fig.
Both Hosts								
Cortinarius spp.	1/SA	1/SA	N/A	2.13a	1/SA	1/SA	N/A	2.13b
Hebeloma spp.	SA, 1/SA	SA, 1/SA	N/A	2.13c	SA, 1/SA	SA, 1/SA	N/A	2.13d
Piloderma spp.	1/SA, SBZ	1/SA, SBZ	reduced by ≈ 50%	2.14a	1/SA, SBZ	1/SA, SBZ	reduced by ≈ 50%	2.14b
Russula spp.	1/SA, SBZ	1/SA, SBZ	reduced by ≈ 65%	2.15a	1/SA, SBZ	1/SA, SBZ	reduced by ≈ 70%	2.15b
Russula nigricans	1/SA	1/SA	N/A	2.15c	1/SA	1/SA	N/A	2.15d
Douglas-fir								
Rhizopogon vinicolor-type	1/SA	1/SA	N/A	2.16a	1/SA	1/SA	N/A	2.16b
Suillus lakei	SA, 1/SA	SA, 1/SA	N/A	2.16c	1/SA, IT	None (see Results)	N/A	2.16d
Paper birch								
Lactarius torminosus	SA, 1/SA, SBZ	1/SA, SBZ (see Results)	Reduced to ≈ 0	2.17a	SA, 1/SA, SBZ	SA, 1/SA, SBZ	Reduced to ≈ 0	2.17b
Leccinum scabrum	SA	SA	N/A	2.17c	SA	SA	N/A	2.17d

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Chapter 3 Do soil properties and tree cover variables explain variation in ectomycorrhizal fungal community diversity and structure along a forest chronosequence?[1]

Introduction

Ectomycorrhizae are prevalent in coniferous forests and play an important role in trees' uptake of water and nutrients and resistance to environmental stresses (Smith & Read, 1997). There is evidence that increasing diversity in ectomycorrhizal (ECM) communities has positive effects on host tree productivity under some conditions (Baxter & Dighton, 2001; Jonsson *et al.*, 2001), which is probably because different ECM fungi differ in functional abilities. The realized niches of ECM fungi are determined in part by their dispersal abilities, specificity to host species, uptake and resistance capabilities, competitive abilities, and response to other environmental factors.

There are myriad biotic and abiotic factors which influence ECM communities. The most obvious are ECM tree community composition and structure, which have been shown to be more important in determining ECM community diversity and composition than other environmental variables, such as soil properties and understorey vegetation (Kernaghan *et al.*, 2003; Nantel & Neumann, 1992; Villeneuve *et al.*, 1989). However, Nantel & Neumann (1992) stress that many ECM fungi occur only within a certain range of abiotic factors for a given host range.

Soil properties also affect ECM communities. Nitrogen (N) and phosphorus (P) acquisition and translocation to plant hosts are especially important functions of ECM fungi. ECM communities may vary over natural gradients of these nutrients. Recent work has shown, for example, that ECM diversity generally decreases and community composition changes over long gradients of increasing available N (Avis *et al.*, 2003; Lilleskov *et al.*, 2002; Peter *et al.*, 2001a; Taylor *et al.*, 2000). Douglas *et al.* (2005), in contrast, found no strong evidence that soil properties, including available N and P, correlated with ECM community differences among lodgepole pine and mixed conifer stands. They examined ECM communities in both forest floor and mineral soil layers, however, and measured soil chemical properties

[1] A version of this chapter will be submitted for publication. Twieg BD, Durall DM, Simard SW, Jones MD. Do soil properties and tree cover variables explain variation in ectomycorrhizal fungal community diversity and structure along a forest chronosequence?

only of mineral soil. Kernaghan *et al.* (2003) also found no effects of N or P on ECM communities in boreal mixed forests, but did find reasonable correlations between soil exchangeable cations and *Russula* and *Cenococcum* abundance.

Ectomycorrhizae are often concentrated in the fermentation layer of the forest floor (Perez-Moreno & Read, 2000), probably because of its high nutrient availability. Many ECM fungal taxa also occur in, and even show preference for, mineral horizons (Rosling *et al.*, 2003; Dickie *et al.*, 2002; Taylor & Bruns, 1999). While N and P can be acquired in mineral form by ECM fungi, it is now recognized that some ECM fungi may also have saprotrophic abilities and take up or transform organic nutrients before translocation to host plants (Read & Perez-Moreno, 2003). Conn & Dighton (2000) showed that litter types affect N and P immobilization and ECM fungal community composition. Conn & Dighton (2000) also found that ECM fungal types differed in their acid phosphatase activities, and greater activity was correlated to litter types with greater P immobilization. This is further evidence that ECM fungi and their functions are strongly linked to soil properties.

Stand age strongly affects ECM communities (see Chapter 2) (Smith *et al.*, 2002; Visser, 1995), but has variable effects on soil properties. Available N and N mineralization can be higher in young stands than in older stands (Bradley *et al.*, 2002; Thibadeau *et al.*, 2000; Prescott, 1997; Bauhus, 1996), but this is not always the case (Kranabetter & Coates, 2004; Griffiths & Swanson, 2001; Barg & Edmonds, 1999; Houston *et al.*, 1998). Organic phosphorus can also be more abundant in recently disturbed than mature stands (Qualls *et al.*, 2000), or may not differ (Kranabetter & Coates, 2004). The C:N ratio of the forest floor can decrease after clearcutting (Olsson *et al.*, 1996), which is likely to affect nutrient cycling (Prescott, 2002). Although models of total organic matter in forest floors often predict losses from the time of forest harvest up to about 20 years of age, the presence and extent of this loss varies substantially (Yanai *et al.*, 2003).

The objective of this study was to examine relationships between soil properties and ECM community measures along a chronosequence of mixed forest stands that were similar in vegetation composition and site quality. The following hypotheses were tested: 1) soil N and P availability and mineralizable N decrease with stand age, while the C:N ratio increases; 2) soil variables explain a substantial degree of variation in ECM diversity that is not accounted for by stand age; namely, inorganic N and P availability are negatively correlated with ECM fungal diversity, while organic P and C:N ratio are positively correlated; and 3) these soil variables are related to ECM community composition and structure.

Materials and Methods

Data Collection and Soil Analyses

Site attributes and methods for characterising the ECM community are detailed in Chapter 2. Six stand types dominated by Douglas-fir and paper birch were selected to represent important seral stand development stages in southern Interior Cedar-Hemlock forests of British Columbia; these were wildfire-origin sites that were in 5-, 26-, 65-, and 100-yr-old age classes, and clearcut-origin sites that were in 5- and 26-yr-old age classes. There were 4 replicate sites selected for each of these six stand types. Total crown cover, and cover of tree species or groups, was estimated along four parallel transects, spaced approx. 5-6 m apart. Canopy cover was estimated for the following groups: 1) Douglas-fir; 2) paper birch; 3) other ECM conifer hosts; 4) other ECM broadleaf hosts; and 5) western redcedar. Percent cover of understorey ECM conifers and western redcedar was also estimated along the transects.

Soil samples were collected in August, 2004. Approximately 1 kg of mineral soil and 300 g of forest floor were removed from each of eight sampling locations at each site. Soil sampling locations were adjacent to four spring and four fall ECM sampling locations. At the 5-yr-old wildfire sites, however, soils were sampled next to eight randomly selected Douglas-fir seedlings located within 3 m of a paper birch tree. No forest floor samples were taken from these burned sites due to the near absence of forest floor layers. Mineral and forest floor samples were bulked separately for each site, separated into three subsamples, sealed in polyethylene bags, and transported on ice in coolers to the lab. Forest floor thickness was measured at each soil sampling location for ectomycorrhizae in spring and fall (see Chapter 2), yielding 16 observations per site.

Mineral soil samples were sieved to 2 mm, air-dried, and sent to the BC Ministry of Forests (MOF) Analytical Chemistry Laboratory in Victoria, BC for all soil analyses except organic phosphorus. Forest floor samples were air-dried and then milled prior to analysis. Total C and N were determined by combustion elemental analysis, using a Leco CHN-600 Elemental Analyser (Leco Corp., St. Joseph, MI, USA) (method described in instrument instructions). Available ammonium and nitrate were extracted by shaking for 2 hours in 2N KCl (Bremner, 1996) and their concentrations measured on a Technicon AutoAnalyser II. Potentially mineralizable ammonium and nitrate were estimated by anaerobic incubation (Waring & Bremner, 1964), done in waterlogged conditions at 30° C for two weeks. Ammonium and nitrate were extracted as above, and initial available N values were subtracted from post-incubation values to estimate mineralizable N. Available P was determined using the Bray-1 method. Organic phosphorus of the forest floor was estimated from the difference in sulphuric acid-extractable phosphorus between post-ignited and pre-ignited soil samples, as described in Page *et al.* (1982).

Measurements were averaged from five readings on the spectrophotometer for each sample. Organic phosphorus analysis was performed at the University of British Columbia, Okanagan.

Data Analysis

Statistical analyses were carried out in SAS v. 9.1 (SAS Institute, Carey, NY, USA) and significance for all statistical tests was set at $\alpha = 0.05$ unless otherwise noted. One way analyses of variance (ANOVAs) were used to test for differences among stand age-initiation types for all soil variables. These analyses were performed on averages of the three subsamples per replicate site. Stand type means were separated using Bonferroni multiple comparison tests. ANOVA assumptions were checked as described in Chapter 2, and where necessary, data were transformed by the natural log.

Five-year-old burned stands were omitted from the rest of analyses because ectomycorrhizae were sampled differently. ECM fungal species richness and evenness were calculated in PC-Ord v. 4 (McCune & Mefford, 1995-2002) for forest floor and mineral soil layers separately, and at both the individual soil sample- and site-levels. Paired t-tests were used to compare site-level species richness and evenness between the forest floor and mineral soil. Generalized linear models, based on maximum likelihood and the Poisson distribution, were used to predict per soil sample ECM species richness. All possible combinations of the following predictor variables were used: stand age, 1/stand age, number of tips identified from the forest floor and mineral soil (separately), and forest floor thickness. AIC was used to select the best model from the resulting set of candidate models (Burnham & Anderson, 2002).

Relative abundance of the most abundant taxa (see Chapter 2) were calculated for mineral and forest floor layers by dividing the total number of tips of each taxon in each layer by the total number of ECM tips examined from each site on the appropriate host(s). Two-tailed paired t-tests were used to determine whether taxa had higher relative abundance in one of the two layers. Sites that had no occurrences of the concerned taxon were removed from this analysis because lack of differences between soil layers is uninformative in circumstances where a taxon is not detected.

Stepwise least-squares multiple regression was used to explore relationships between soil variables and ECM diversity. To do this, site scores for the first Principal Component of a Principal Components Analysis (PCA) on ECM fungal diversity variables (see Chapter 2), species richness in the combined community (i.e. both hosts), and species richness on Douglas-fir were predicted from the following variables for mineral soil and forest floor: C:N ratio, available N, potentially mineralizable N, and available P. Stand age, biogeoclimatic subzone, forest floor organic P, and site index were also used

as predictor variables. The criteria for entry and retention in the regression models were 0.15 and 0.1 for F-test and partial F-test significance, respectively. Stepwise regressions were also used to predict relative abundance of *Cenococcum geophilum*, *Rhizopogon vinicolor*-type, and *Russula* spp. using the same methods and predictor variables as for site-level ECM diversity models. These taxa were chosen because of their high frequency and abundance, and because their relative abundance data met normality and homoscedasticity assumptions.

It was originally intended that ECM variables would be regressed against tree cover variables, but each had 7-10 zero values out of the 20 sites, rendering these data inappropriate for multiple regression analyses. The ranges of values in tree cover variables were also fairly low (see Table 3.1) because the sites were intentionally selected to be relatively pure mixtures of Douglas-fir and paper birch. A Mantel Test was therefore used to determine whether site similarity in the ECM community was related to site similarity in tree cover variables. Frequency of ECM fungal species in the combined community was used in one matrix, and the tree cover variables presented in Table 3.1 were used in the other matrix. The Relative Sorensen distance measure was chosen for the ECM community matrix and Sorensen distance for tree cover, and tests based on both Monte Carlo randomizations (1000) and Mantel's Asymptotic *z* Approximation were checked for significance of the intermatrix correlation. This analysis was performed both with and without 5-yr-old sites included because root systems of other tree species in these stands were much less likely to overlap those of target hosts and were therefore less likely to affect their ECM fungal communities. Another analysis was done with Douglas-fir and paper birch cover removed from the tree variables matrix to get an idea of how much other trees were related to ECM communities on the target hosts.

Canonical Correspondence Analysis (CCA) in PC-ORD was used to ordinate sites and ECM fungal species based on species frequencies (the number of soil samples each species occurred in per site). The same soil variables used as independent variables in regressions predicting diversity PCA scores were used as the environmental matrix. Only species that occurred in more than two sites were included. Monte Carlo randomisations (1000) were used to test significance of site ordination and correlation between the environmental variables and ECM communities. Stepwise regression analysis was also used with stand age and soil variables as predictors for site scores from a well-structured NMS ordination of the entire ECM community (see Chapter 2).

Results

Mineral soil C:N ratio differed among stand types, mostly due to higher C content in 26-yr-old clearcut stands (Table 3.2). C:N ratio of the forest floor was 40% higher in 5-yr-old clearcut stands than 100-yr-old stands because of lower total N in the 5-yr-old stands. Total C content of the forest floor was similar among stand types (range 34.4-38.5%). Stand types differed in available nitrate of the mineral soil and available ammonium of the forest floor, but multiple comparison tests revealed no pairwise differences. Mineral soil mineralizable nitrate in 26-yr-old sites was about twice as high as in 65- and 100-yr-old sites, but overall, mineralizable nitrate averaged only 5% of mineralizable ammonium levels (range 3.1-10.5%). Stand types did not differ in any of the other soil parameters measured.

Forest floor thickness was similar among stand types (Figure 3.1). The total number of root tips included in site-level analyses was also similar for all sites (see Chapter 2), as were the cumulative numbers of ECM fungal species detected in the mineral soil and forest floor layers (73 and 82, respectively). Mean site-level species richness, however, was 27% higher in the forest floor than the mineral soil (Fig. 3.2). The average number of root tips examined from the forest floor was also about 30% higher than the number examined from mineral soil. A frequency distribution comparing the number of tips examined per core between soil layers shows that more mineral soil samples had fewer than 50 ECM root tips than did forest floor samples (141 vs. 99, respectively; Fig. 3.3). The ratio of the number of root tips examined from the forest floor layer to mineral soil varied considerably, but was not related to site-level diversity, nor did it differ among stand types (Fig. 3.4). Site-level evenness of the ECM community did not differ between soil layers.

The best models predicting per soil sample ECM richness of Douglas-fir and the combined community included stand age (Fig. 3.5) and the number of root tips examined from the forest floor; neither the number of tips from the mineral soil nor forest floor thickness was a significant predictor. Including forest floor root tip number increased soil sample-level prediction of ECM species richness on Douglas-fir and the combined community by about one species per 10 root tips.

Fifty-five of the 105 identified species of ECM fungi were found in only one of the two soil layers, but these species were too infrequent to evaluate their soil layer preference. Several frequently observed ECM taxa occurred in both layers, but showed preference for one layer. *Leccinum scabrum*, *Rhizopogon rudus*, and *Suillus lakei* were more abundant in the mineral soil, while *Lactarius torminosus* and the genera *Cortinarius*, *Hebeloma*, and *Piloderma* were more abundant in the forest floor (Table 3.3).

Cenococcum geophilum, *Lactarius pubescens*, *Lactarius scrobiculatus*, and the genus *Russula* did not differ in relative abundance between soil layers.

Models predicting site-level ECM richness and diversity are summarised in Table 3.4. As detailed in Chapter 2, stand age and sometimes biogeoclimatic subzone were significant predictors of ECM species richness and diversity. Forest floor organic P was positively related to ECM diversity PCA axis one scores and ECM diversity on Douglas-fir. Mineral soil available P was negatively related to diversity on Douglas-fir. However, when one site that had the lowest ECM fungal richness and diversity and second-lowest organic P value (555 mg/kg soil) was removed from these analyses, no soil variables were significant predictors, but stand age and biogeoclimatic subzone remained significant. This site was an outlier, being the only site with richness and diversity values for the ECM fungal community more extreme than two standard deviations from overall mean values. Diversity is plotted against organic P in Figure 3.6, including the outlier site.

There were some significant relationships between soil variables and relative abundances of dominant ECM taxa. Mineral soil available N and P and forest floor available P were significant in models predicting *Cenococcum geophilum* relative abundance, and mineral soil available N and P were also significant in predicting *Rhizopogon vinicolor*-type relative abundance (Table 3.5). Out of the variables that were significant in predicting *Cenococcum geophilum and Rhizopogon vinicolor*-type relative abundances from both soil layers combined, only forest floor available P remained significant in regressions predicting abundances from each soil layer separately.

Tree cover variables (% canopy cover of Douglas-fir, paper birch, other ECM broadleaves, other ECM conifers, and western redcedar, and understorey cover of other ECM conifers and western redcedar) were fairly well correlated with ECM community. The relationship was stronger with 5-yr-old stands included (Mantel's Standardized $r = 0.44$; $p = 0.00001$ and 0.001 for Mantel's Asymptotic Approx. and Monte Carlo tests, respectively) than when they were removed ($r = 0.25$; $p = 0.016$ and 0.007). Correlation values ($r = 0.39$ with and 0.30 without 5-yr-old sites) and significance were similar with Douglas-fir and paper birch cover removed from the analysis.

Roughly half of the ECM fungal species occurred in two or fewer sites, and were removed for CCA. Sites grouped strongly by stand type in the ordination (Fig. 3.7), but ordination structure was better than random permutations of the data on only one axis (Table 3.6). While site index and mineral soil C:N ratio and available P had moderate correlations to one axis each, their correlations were not nearly as strong as that of stand age. Overall correlations between species and environmental matrices were not

significant. Similarly, no soil variables were correlated to axis scores from a well-structured NMS ordination of the combined community, nor was there a significant correlation between the ECM community and soil variable matrices (Mantel's Standardized $r = 0.10$; p-values = 0.32 and 0.18 for Mantel's Asymptotic Approx. and Monte Carlo tests, respectively).

Discussion

The data did not support the first hypothesis that mineral forms of N and P are more available, and C:N ratio lower, in younger stands. Indeed, general patterns in C, N, P, or forest floor depth across stand ages were not evident. Although N mineralization is commonly higher in recent clearcuts or forest gaps than in mature forests, this effect is most pronounced in the first year following disturbance and subsequently tapers off over the next few years (Prescott, 2002; Bauhus & Barthel, 1995). In this study, an early N flush would have been missed because the youngest age class sampled was 5 years old. Consistent with this, Kranabetter & Coates (2004) found no difference in soil available N and P, or organic P between mature ICH stands and 10-yr-old plantations. Forge & Simard (2000), however, found that mineral N was lower in 10 yr-old plantations than adjacent mature ICH forests, probably because of uptake by the lush herbaceous vegetation layer that had developed after harvest. Forest floor mineralizable N levels found by Forge & Simard (2000) were generally lower than, but not outside of the range of, those found in this study. The overall difference is surprising, given the geographical proximity of these two studies, but it may have been caused by sampling at different times of year; Forge & Simard (2000) sampled in June and September, while samples were taken in August in the current study. Soil N and P values in this study are similar to Kranabetter & Coates (2004), who examined western hemlock-dominated sites of consistent quality in the ICH of northern British Columbia.

There was considerable variation in soil properties among the sites, even though all were mesic (zonal site series; (Lloyd *et al.*, 1990)). Our data did not strongly support the second hypothesis that soil nitrogen, phosphorus, and C:N ratio explain a high degree of the variation in ECM diversity. Relationships between diversity and both available and organic P were largely the result of one site that was at the low end of the range of organic P levels encountered in this study. A study concentrating on more sites within the lower range of organic P values would be necessary to confirm or refute this relationship. In this study, there were only two sites with levels of organic P that were below two standard deviations of the overall mean, and both sites were from the youngest age class. Soil N was not related to diversity. Although Lilleskov *et al.* (2002) found that below-ground ECM diversity was negatively related to extractable (available) mineral N in the forest floor, their nitrogen gradient was extreme compared to this study. Avis *et al.* (2003) and Peter *et al.* (2001a) both found that nitrogen addition affected species richness and diversity of the ECM sporocarp communities more than root tip communities. Lilleskov & Bruns (2001) suggest that nitrogen fertilization may simply reduce the amount of resources allotted to fruiting without having a prominent effect on below-ground community structure.

The hypothesis that soil nutrients are related to ECM community structure was supported by some specific taxa in this study. Although soil variables did not greatly improve relative abundance models, two out of three dominant ECM fungal taxa were positively related to available forest floor phosphorus. This study cannot determine whether higher available P caused higher ECM abundance of certain taxa or vice-versa, but it is known that ECM fungi can access organic P and make it available to plants (Sawyer *et al.*, 2003) and that ECM fungi can reduce levels of organic P more substantially than available P from forest floor material (Perez-Moreno & Read, 2000).

It is somewhat surprising that soil N was not strongly related to abundance of dominant ECM taxa. *Cenococcum* relative abundance from both soil layers combined was positively related to available N of the mineral soil only, but this relationship was not apparent when only its abundance in the mineral soil was considered. This was not surprising given available N was much higher in the forest floor than mineral soil, that *Cenococcum* was unrelated to forest floor N, and that this taxon showed no mineral soil versus forest floor preference. Nilsen *et al.* (1998) also found no effect of N on *Cenococcum* relative abundance on root tips. Nevertheless, available N in the mineral soil could have some effect on colonisation in both soil layers by influencing exploratory patterns of *Cenococcum* hyphae, or indirectly by influencing plant and microbial communities. Although below-ground *Russula* abundance has been shown to decrease (Peter *et al.*, 2001a) or increase (Avis *et al.*, 2003) following N-fertilization, it was not related to N levels in this study. Changes in *Russula* abundance in these two studies were largely due to one *Russula* species each, and effects of higher N availability in fertilized treatments in the study of Avis *et al.* (2003) was somewhat confounded by simultaneously higher available P.

While ECM community structure was reasonably correlated with tree cover variables, there was generally no relationship between ECM community structure and soil variables. Mantel correlations suggested that tree cover of species besides Douglas-fir and paper birch was less correlated to ECM communities of these two hosts in 5-yr-old stands than in older stands. This is not surprising, given that tree root systems are far more developed in older stands and are therefore more likely to interact with each other. CCA ordinations of sites based on ECM community and environmental variables only showed a strong correlation of ECM communities and stand age. NMS ordination (see Chapter 2) accounted for much more variation in ECM community structure than did CCA. This may be attributed partly to the fact that the implicit Chi-square distance measure in CCA gives higher weight to rare species than the Relative Sorensen distance measure that was applied in NMS (McCune *et al.*, 2002), but also indicates that the measured soil variables were not related to ECM communities in predictable ways. The fact that these soil variables were not significantly related to site scores on NMS axes or significantly

correlated to ECM communities is further evidence that they had little effect on ECM community structure.

Although total ECM root tips in the mineral soil and forest floor were not directly counted, they appeared denser and more regularly distributed in the forest floor than mineral soil. The overall preference of ectomycorrhizae for the forest floor over the mineral soil was particularly apparent because a much higher volume of mineral soil than forest floor was examined on average per sample, and yet there were many more mineral soil samples than forest floor samples with fewer than fifty ECM root tips. ECM tips have previously been shown to be concentrated more in the forest floor than mineral soil (Nelville *et al.*, 2002; Erland & Taylor, 2002), which probably reflects greater resource availability and heterogeneity in the forest floor. Site-level ECM richness was not substantially higher in the forest floor than the mineral soil, although richness was significantly higher in the forest floor at the soil sample-level. The high ECM fungal diversity and preference of some ECM fungal species for mineral soil suggests that both forest floor and mineral soil require sampling to accurately characterise ECM communities.

Conclusions

While soil properties were not related to ECM community structure at the site level in this study, they may affect ECM composition at very small spatial scales (Schimel & Bennett, 2004). As suggested by recent studies, total soil organic content and mineral forms of N and P are likely not as closely related to ectomycorrhizae as are organic nutrient complexes. Other soil attributes, such as pH, soil moisture variation over time, and micronutrients may also play important roles in ECM community structure. Differences in inoculum availability and non-ECM plant community structure were likely responsible for much of the variation in ECM diversity and community composition that were not accounted for by stand age.

Figures

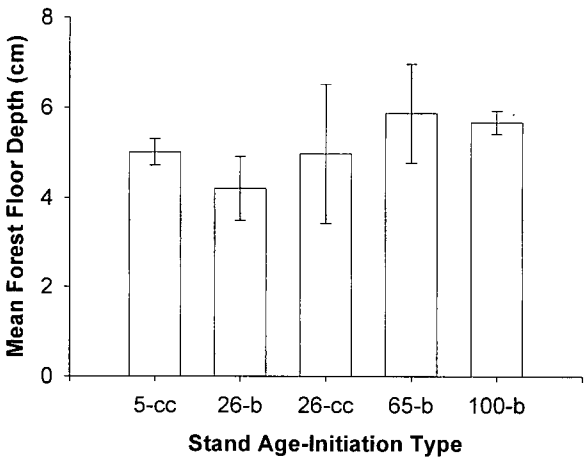

Figure 3.1 Mean forest floor thickness (n = 4) by stand age-initiation type; cc = clearcut; b = burned. Error bars represent one standard error of the mean. No differences were detected by one-way ANOVA (F = 1.26, p = 0.29).

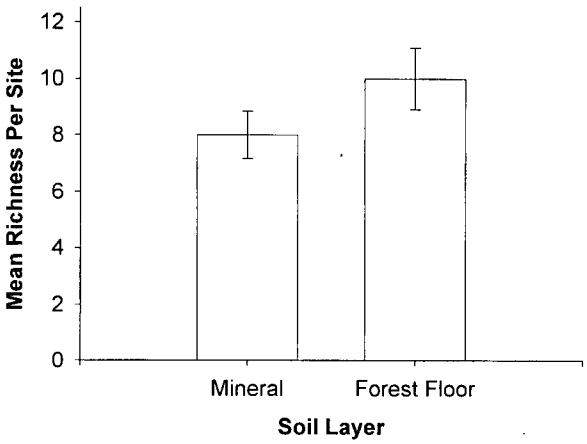

Figure 3.2 Mean ECM fungal species richness of the community of both hosts in mineral soil and forest floor layers. Error bars represent one standard error of the mean. Significant difference detected by paired t-test ($n = 20$, $t = -3.02$, $p = 0.007$).

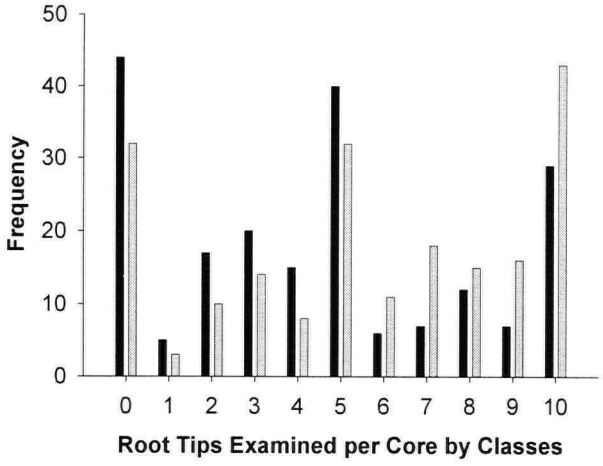

Figure 3.3 Frequency histogram of number of ECM rot tips available per soil sample from mineral soil (black bars) and forest floor (grey bars) (to max. of 100 tips). 0 = 0 tips examined; 1 = 1-10 tips; 2 = 11-20 tips ...; 10 = 91-100 tips examined.

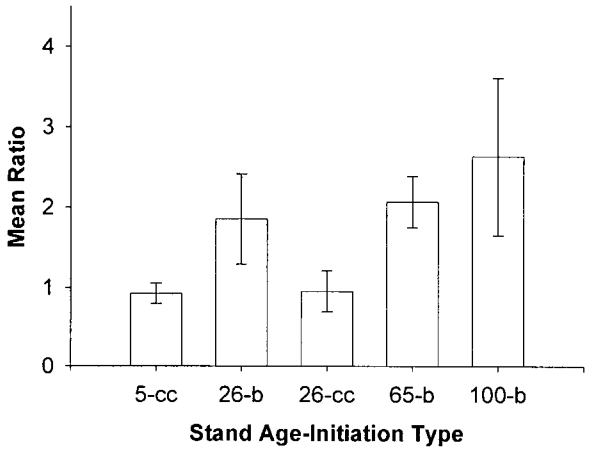

Figure 3.4 Mean ratio of number of root tips examined from the forest floor to the number examined from the mineral soil per site (n = 4). Error bars represent one standard error of the mean. No significant differences detected by one-way ANOVA (F = 1.9, p = 0.17).

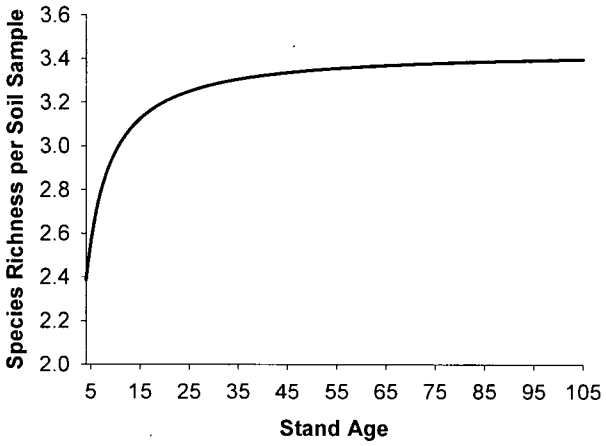

Figure 3.5 Maximum likelihood model (using Poisson distribution) prediction of ECM fungal species richness per soil sample by stand age. Number of root tips examined from the forest floor was also a significant predictor variable in this model (see Results). Stand age was a significant predictor (likelihood ratio test; $p < 0.05$).

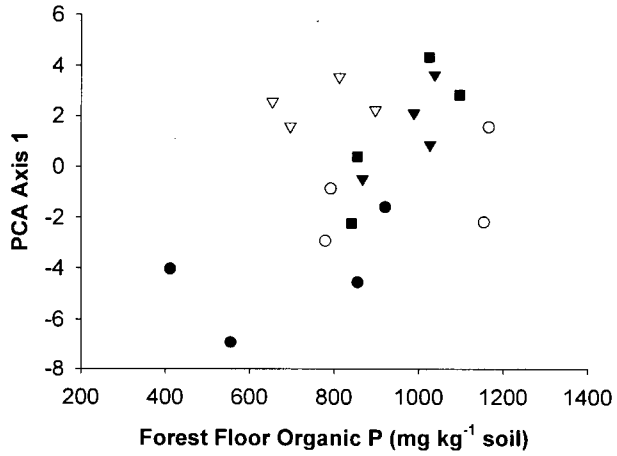

Figure 3.6 Scatterplot of principal component axis 1 site scores (from PCA on 13 ECM diversity variables) against forest floor organic P; filled circles = 5-yr-old clearcuts, open circles = 26-yr-old wildfire origin, filled triangles = 26-yr-old clearcuts, open triangles = 65-yr-old wildfire origin, squares = 100-yr-old wildfire origin.

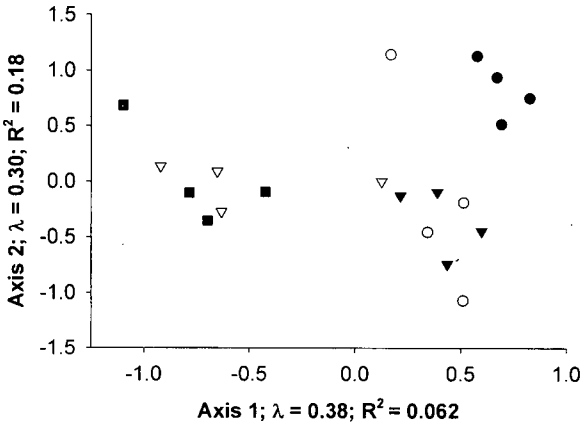

Figure 3.7 CCA ordination of sites based on frequency of ECM fungi in the combined community of both hosts and environmental variables (stand age, site index, and soil variables). Filled circles = 5-yr-old clearcut; open circles = 26-yr-old burned sites; filled triangles = 26-yr-old clearcut sites; open triangles = 65-yr-old burned sites; and squares = 100-yr-old burned sites. λ = axis eigenvalue; R^2 = proportion of variance in Chi-squared distance among sites explained by ordination axes.

Tables

Table 3.1 Tree cover variables by site; Fd = Douglas-fir, Ep = paper birch.

		Percent Canopy Cover					% Understorey Cover – Other ECM Conifers	% Understorey Cover - Western redcedar
Site	Age	Fd	Ep	Other ECM Conifers [1]	Other ECM Broadleaves [2]	Western redcedar		
19MR	6	5	33	8	1	4	0	0
AL	6	11	23	8	3	0	0	0
BC	4	3	55	3	3	0	0	0
WL	5	5	35	9	1	1	0	0
ED1	30	28	32	6	10	0	8	8
ED2	30	30	38	9	4	0	3	3
MA1	24	28	32	4	7	0	13	8
MA2	24	32	28	5	5	0	8	3
DISC	27	26	39	0	0	0	0	0
NM	21	24	27	0	3	6	13	8
SRC	22	36	38	0	2	0	0	3
ZP	25	28	28	7	7	0	8	3
BA	63	38	43	4	0	4	0	3
MARA	71	36	40	0	0	4	3	8
RR	61	30	45	2.5	0	0	8	0
SL	68	41	41	5	2	2	0	8
4WD	103	45	41	0	0	5	0	0
ACR	98	44	24	6	0	6	0	13
BBP	101	32	32	5	0	8	8	3
WAP	90	40	32	0	0	8	3	8

[1] Includes hybrid spruce, western hemlock (canopy in 5-year-old stands only; understorey in older stands), western white pine (*Pinus monticola* Dougl. Ex D. Don in Lamb.), and lodgepole pine

[2] Includes black cottonwood, trembling aspen, and willow spp.

Table 3.2 Mean soil properties by stand age and initiation type (n = 4; standard error of mean in parentheses). Means followed by the same letter (within one soil layer and variable) are not significantly different (p > 0.05) by multiple comparisons. § = difference among treatment means, but no significant differences found in pairwise mean comparisons. F-ratios and p-values are from one-way ANOVAs.

Stand Age and Initiation Type	C/N Ratio	Available Ammonium	Available Nitrate	Mineralizable Ammonium	Mineralizable Nitrate	Organic P	Available P
Mineral Soil							
5-yr-old Burned	23.2 (0.55)a	2.60 (0.42)	0.18 (0.06) §	18.3 (1.3)	0.678 (0.149)ab	N/A	143 (24)
5-yr-old Clearcut	29.9 (1.48)ab	1.97 (0.18)	0.33 (0.03)	15.3 (1.8)	0.730 (0.145)ab	N/A	159 (19)
26-yr-old Burned	29.6 (1.94)ab	2.23 (0.51)	0.38 (0.06)	14.4 (2.8)	0.974 (0.128)b	N/A	208 (49)
26-yr-old Clearcut	35.0 (1.13)b	2.44 (0.38)	0.28 (0.04)	9.2 (3.2)	0.971 (0.111)b	N/A	95 (16)
65-yr-old Burned	24.6 (0.56)a	2.18 (0.33)	0.38 (0.02)	14.8 (2.7)	0.490 (0.114)a	N/A	139 (18)
100 yr-old Burned	25.3 (0.79)ab	2.02 (0.27)	0.30 (0.02)	14.1 (1.3)	0.442 (0.128)a	N/A	209 (24)
F-ratio	4.11	0.45	3.00	1.68	5.28	N/A	0.88
P-value	0.0115	0.8108	0.0386	0.1914	0.0037	N/A	0.5162
Forest Floor							
5-yr-old Clearcut	47.0 (2.55)b	11.7 (2.5)§	0.39 (0.23)	230 (29)	1.111 (0.193)	685 (121)	101 (11)
26-yr-old Clearcut	38.3 (1.17)ab	27.1 (3.5)	0.95 (0.22)	375 (22)	1.570 (0.344)	973 (108)	133 (16)
26-yr-old Burned	34.1 (1.80)ab	22.8 (2.3)	0.58 (0.14)	440 (34)	1.660 (0.310)	980 (39)	150 (7.4)
65-yr-old Burned	35.2 (1.58)ab	18.4 (3.8)	0.83 (0.34)	309 (29)	1.512 (0.246)	765 (55)	112 (8.6)
100-yr-old Burned	33.7 (1.05)a	26.9 (4.8)	1.0 (0.39)	411 (48)	1.363 (0.219)	954 (63)	102 (6.2)
F-ratio	3.09	3.40	0.93	1.94	0.54	2.67	1.24
P-value	0.0485	0.0362	0.4717	0.1557	0.7807	0.0729	0.3357

Table 3.3 Mean relative abundances (respective of host) of dominant taxa by soil layer. Fd = Douglas-fir; Ep = paper birch. Significant differences have p-values in bold (paired t-test).

ECM Taxon	Host Tree	Number of Sites (n)	Mineral Soil Relative Abundance (%)	Forest Floor Relative Abundance (%)	P-value: Paired T-test
Rhizopogon vinicolor-type	Fd	20	18.0	23.5	0.266
Rhizopogon rudus	Fd	6	8.95	2.39	**0.026**
Suillus lakei	Fd	9	14.9	2.86	**0.016**
Lactarius pubescens	Ep	5	8.84	7.48	0.759
Lactarius torminosus	Ep	10	2.74	12.1	**0.033**
Leccinum scabrum	Ep	16	8.70	1.58	**0.012**
Cenococcum geophilum	Both	20	4.11	6.15	0.059
Cortinarius spp.	Both	16	0.91	4.40	**0.030**
Hebeloma spp.	Both	11	1.43	4.79	**0.003**
Lactarius scrobiculatus	Both	11	1.96	4.24	0.245
Piloderma spp.	Both	14	0.89	7.68	**0.002**
Russula spp.	Both	18	7.08	6.66	0.881

Table 3.4 Summary of stepwise regression analyses predicting ECM diversity variables. R^2 values in bold correspond to the model that contains all significant predictor variables; those not in bold correspond to model including that predictor variable and those above it for the corresponding dependent variable.

Y-variable/ x-variables	Range of Y-variable	Range of X-variable	Slope or Intercept Estimate	Partial F-test P-value	R^2	Model F-test P-value
Diversity Principal Component	-6.92 to 4.35					0.0001
1/stand age		.009 to 0.17	-27.407	0.0004	0.51	
Subzone		0 (ICHmw); 1 (ICHmk)	-4.148	0.0074	0.65	
Forest Floor Organic P		412 to 1166	0.0043	0.0590	**0.72**	
Intercept		N/A	2.054	0.3703		
Richness of Combined Community	9 to 27					0.0010
1/stand age		See above	-46.397	0.0006	0.40	
Subzone		See above	-6.44	0.0246	**0.56**	
Intercept		N/A	21.991	<0.0001		
Richness of Douglas-fir Community	2 to 16					0.0001
Stand age		4 to 103	-0.0885	<0.0001	0.52	
Forest Floor Organic P		See above	0.00771	0.0177	0.62	
Mineral Soil Available P		58 to 484	-0.0131	0.0294	**0.72**	
Intercept		N/A	0.9157	0.7279		

Table 3.5 Summary of stepwise regression models predicting relative abundances (%) of dominant ECM taxa. R^2 values as in Table 3.4.

Y-variable/ x-variables	Range of Y-variable	Range of X-variable	Slope or Intercept Estimate	Partial F-test P-value	R^2	Model F-test P-value
Cenococcum geophilum	2.9 to 18.8%					0.0005
Mineral Soil Available N		1.55 to 3.94	4.383	0.0005	0.41	
Forest Floor Available P		45 to 195	0.0563	0.0100	0.51	
Mineral Soil Available P		58 to 484	-0.0200	0.0152	**0.66**	
Intercept		N/A	-4.367	0.1696		
***Rhizopogon vinicolor*-type**	5.3 to 89.1%					<0.0001
1/stand age		.009 to 0.17	295.11	<0.0001	0.64	
Forest Floor Available P		See above	0.1570	0.0695	0.74	
Mineral Soil Available P		See Above	0.0568	0.0770	**0.79**	
Intercept		N/A	-4.09	0.6898		
Russula spp.	0 to 37.8%					<0.0001
Stand age		See above	0.2749	<0.0001	**0.68**	
Intercept		N/A	0.6974	0.7787		

Table 3.6 Details of CCA ordination of sites based on ECM fungi frequency in the combined community and environmental variables. Correlations of environmental variables to ordination axes are Pearson's *r*, and those of 0.5 or above are in bold. R^2 is the proportion of Chi-square distance among sites that is explained by ordination axes. P-values for Monte Carlo test of ordination structure represent the proportion of randomized sets of the real data giving ordination axes with axis eigenvalues greater than or equal to the real data. Those for tests of inter-matrix correlation represent the proportion of randomized data sets giving correlations between ECM species matrices and the environmental variables equal to or greater than the real data.

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.378	0.302	0.257
R^2	0.062	0.184	0.015
P (Monte Carlo test): Ordination structure	0.008	0.122	0.360
P (Monte Carlo test): Inter-matrix correlation	0.057	0.515	0.243
Correlations			
Stand Age	**-0.919**	-0.201	-0.234
Site Index	-0.358	-0.019	**-0.558**
Mineral Soil			
C:N ratio	**0.533**	0.187	0.249
Available N	0.066	-0.026	-0.356
Mineralizable N	-0.080	0.254	-0.283
Available P	-0.242	**0.523**	0.003
Forest Floor			
C:N ratio	0.438	0.274	0.374
Available N	-0.185	-0.366	-0.220
Mineralizable N	-0.018	-0.441	-0.236
Available P	0.023	0.063	0.218
Organic P	-0.088	-0.248	0.103

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Chapter 4 Conclusions

Ectomycorrhizal Diversity and Communities

Effects and Likely Causes

The 5-year-old clearcuts supported less diverse ECM communities than older stands, which is consistent with many other studies (Durall *et al.*, 2006; Hagerman *et al.*, 2001; Byrd *et al.*, 2000; Hagerman *et al.*, 1999a; Durall *et al.*, 1999; Kranabetter & Wylie, 1998). Reductions in diversity after disturbance are partly caused by poor inoculation of seedlings establishing in isolation of mature trees or forest (see Cline *et al.*, 2005; Hagerman *et al.*, 1999a). There was little evidence that lower ECM diversity in young stands was associated with available forms of N or P, organic P, or C:N ratio. Although high mycorrhizal diversity is often correlated with low nutrient availability, this did not appear to be the cause of higher ECM diversity in older stands. While effects of non-ECM plants on ectomycorrhizae were not examined here, young stands were dominated by shade intolerant shrubs and herbs rather than ECM conifers.

Age of host trees also affects ECM communities (Jones *et al.*, 2003). The rare occurrence in young stands of some fungi common in older stands suggests that some of these fungi (e.g. *Piloderma fallax*, *Russula nigricans*, *Lactarius rubrilacteus*, and Phallales 1) are physiologically able to form mycorrhizae with very young trees, but do not compete well in the clearcut environment. As suggested by Kranabetter & Friesen (2002), these fungal species may require connections to mature trees; although lack of mature trees may not preclude them from establishing on seedlings, greater available carbon from older trees may enhance their competitive abilities on seedling root tips.

The greater ECM community diversity on paper birch than Douglas-fir in the youngest stands was striking. The stump sprouting habit of birch allows many of its roots to continue living after stems have been cut, providing pre-disturbance inoculum for establishing seedlings. Retaining living birch stumps would therefore mitigate inoculum loss that occurs following clearcutting (Hagerman *et al.*, 1999b; Hagerman *et al.*, 1999a) and wildfire (Grogan *et al.*, 2000; Baar *et al.*, 1999). In my study, it is likely that some Douglas-fir inoculum was lost between the time of clearcutting and planting. Furthermore, ECM community composition of paper birch in young stands was still different from older

stands. Clearcutting affects more than just inoculum levels, and results in changes to host age, non-ECM host plant communities, microbial communities, and environmental conditions and resources, all which can affect the ECM of establishing hosts.

Implications

Although I could not determine the cause of lower diversity in young stands, I feel there are clear land management implications from this study. Based on the taxa-sampling unit curves, it appears that the site-level difference in diversity between the 5-year-old and older stands was underestimated, and that landscape-level richness is probably several times lower in an equivalent area of 5-year-old forest than in mature forest. There was ample inoculum in young stands to facilitate complete colonisation of seedlings, but lower ECM diversity in young stands will reduce the diversity of inoculum available on a landscape level if a large proportion of young forest is maintained. While this reduction in diversity of available inocula may not cause a reduction in species diversity in the short term, there is high potential for loss of genetic diversity.

Another obvious implication is that removal of paper birch from young stands will decrease stand-level ECM diversity. Although the effect of stump sprouts on ECM diversity was not directly tested in this study, my results suggest that living roots of sprouting stumps link one forest generation to the next by providing inoculum for newly establishing hosts. Conversely, removal of birch stumps after logging for mitigation of *Armillaria ostoyae* root disease or for reduction of competition with conifers may reduce ECM diversity during stand initiation.

Despite the beneficial contribution of paper birch to total ECM diversity in young stands, it appears unlikely that it contributed to Douglas-fir ECM communities because of the dominance of host-specific fungi on Douglas-fir in 4- to 6-year-old stands. This was probably not due to more limited contact between roots of paper birch with those of Douglas-fir, because roots of both hosts were commonly found in the same soil samples in the 5-yr-old stands as well as the old stands. However, compared with Douglas-fir, paper birch accumulated a higher proportion of ECM species compatible with both hosts in earlier age classes. Hence, it likely played an important role in determining ECM community structure on Douglas-fir over the chronosequence.

Succession Models and Fungal Strategies

The ECM fungal patterns observed in this study are only partially consistent with historical models recognizing only two or three categories ("early-, multi-, and late-stage") of ECM fungi in forest succession (Visser, 1995; Mason *et al.*, 1983; Fleming, 1983). My results do not support the early dichotomous classification system (Mason *et al.*, 1983), in which "early-stage" fungi dominate young stands, but are replaced by "late-stage" fungi in older stands. *Lactarius pubescens* was the only ECM fungus that was dominant in young stands but absent in older ones, consistent with Visser's (1995) finding of few "early-stage" fungi in young stands. However, earlier research categorized *L. pubescens* as a "late-stage" fungus (Fleming, 1983; Fox, 1983), which appears to disagree with plant and fungal succession patterns in ICH forests. Small gap disturbances, which are common in mature ICH forests, can also result in localized dominance of fungi common in early stand initiation (Kranabetter & Friesen, 2002). Thus, the use of "early-stage/late-stage" terminology should be applied carefully when describing ECM community dynamics in ICH forests.

Fungal species that dominated the youngest stands were not necessarily ruderal strategists because they were previously shown to have substantially reduced inoculation potential when disconnected from parent trees (Simard *et al.*, 1997b; Fleming, 1984). *Rhizopogon vinicolor*-type fungi and *L. pubescens*, for example, likely expend substantial energy inoculating new ECM root tips via vegetative spread rather than concentrating on formation of spores in response to reductions in host availability. It is difficult to fit ECM fungi into plant strategies proposed by Grime (1977) because little is known about which resources (e.g. host roots, soil nutrients and moisture) ECM fungi are competing most strongly for. Low availability of host roots and associated scarcity of carbon in 5-yr-old stands could be the most important resource stress imposed on ECM fungi among the stands studied. *Thelephora terrestris*, often referred to as an "early-stage" or ruderal fungus, occurred only in 5-year-old sites, but its frequency and abundance was low. *Wilcoxina rehmii*, part of the group of fungi forming E-strain mycorrhizae that also tends to proliferate immediately after disturbance, occurred at very low frequency and abundance at all forest ages. These fungi may indeed act as true ruderal strategists, establishing well from spores in the wake of disturbance but apparently lacking the ability to compete after other fungi establish.

This study does support the grouping of several fungi as "multi-stage", as described by Visser (1995) and supported by Smith *et al.* (2002) and Kranabetter *et al.* (2005). Fungi that fall into this category include *Rhizopogon vinicolor*, *R. vesiculosus*, *Amphinema byssoides*, *Cenococcum geophilum*, *Tuber* 1, and some *Inocybe* and *Tomentella* species. Fungi that augmented the community in stands older

than the 5-years were not necessarily "late-stage". For instance, *Russula* and *Piloderma* were nearly absent in 5-year-old stands, then increased to comprise a moderate proportion of the community in 26-year-old stands, and continued to increase in frequency and relative abundance with increasing stand age. Therefore, at the genus level, *Russula* and *Piloderma* fall somewhere between "multi-stage" and "late-stage". Other fungi, such as *Lactarius scrobiculatus*, were mostly absent from 5-year-old stands, but were abundant to similar degrees in all other age classes.

Parallels can be drawn between plant-community succession models and ECM community successional patterns. Examples consistent with both "relay floristics" and "initial floristics" models were observed in this study. *Lactarius pubescens* was largely replaced by *L. torminosus* in older stands, which is consistent with the "relay floristics" concept, but *L. torminosus* was also found once in a 5-year-old site. It is likely that many other taxa common to the older age classes, such as *Russula* and *Piloderma* species, were also present in 5-year-old stands but not detected due to their very low frequency and abundance. This is more consistent with the "initial floristics" model.

It seems that no single model is adequate to describe the complexity of successional patterns of ECM fungi. Using forest stand development stages to help characterize ECM community succession patterns may be more useful than classifying fungi into their own successional categories or trying to fit them into simplified plant succession models. Sites in the stand initiation stage (5-years-old) had distinctive ECM community composition and structure as well as low ECM diversity. Stem exclusion stage sites (26-years-old) had higher ECM diversity, but community structure was intermediate between 5-year-old sites and older sites. For instance, *Rhizopogon vinicolor*-type was much less dominant and *Lactarius pubescens* was almost absent in this age class, while *Russula* and *Piloderma* were significant components of the community. However, other fungi, like *Suillus lakei*, *Hebeloma* spp., and *Cortinarius* spp., were prominent in 26-year-old stands, but were largely supplanted by *Russula* and *Piloderma* in older stands. Sites in the stand re-initiation stage (65- and 100-year-old) were similar to each other, more so than to other age classes, in ECM diversity and community composition and structure.

Study Shortcomings

The most important shortcoming of this study was its use of a chronosequence of sites as a proxy for observing succession on the same sites through time. Variation in ECM communities that was unaccounted for by stand age may have been partially due to the inherent flaws in chronosequences (Simard & Sachs, 2004), and this may have hindered the ability to detect relationships between ECM fungal parameters and soil properties. It cannot be assumed that the history of all replicate stands within the same disturbance type were the same. There were also slight differences in the management regime of clearcuts (e.g. nursery stock, conifer mixtures replanted) because different forest companies were responsible for managing different sites. It was necessary to select replicate stands that were far enough apart to avoid spatial autocorrelation; this may have increased between-site variability. In spite of these shortcomings, chronosequence studies provide a useful assessment of succession without waiting lifetimes for forests to develop.

I found that combining morphotyping and DNA analysis was highly successful at fungal taxon identification. However, several samples could not be included in analyses because more than one fungus was present on a single root tip. In addition, the cost of molecular analysis was too high for analysis of all samples. The morphology of only a small percent of the ECM fungal species that occurred in this study had been previously described, so some subjectivity was necessary in combining molecular and morphological data to arrive at the final data set for analyses. However, a cautious approach was taken, and samples with ambiguous taxonomic placement were omitted from the analyses. In the future, a less rigorous morphotyping approach would allow more resource allocation to DNA analyses, although examination of ECM tips under a compound microscope is still recommended to reduce sorting error.

A more intensive soil sampling approach would have enhanced our ability to correlate soil parameters with ECM parameters, but most project resources went to proper identification of ectomycorrhizae. Given our resources, it was not possible to measure soil properties and ectomycorrhizae within the same core, with many cores sampled over an experimental unit. This might have been useful, since both sets of factors vary at the microsite level. Nevertheless, our sampling intensity for environmental variables allowed us to characterize each study site.

Future Directions

A better assessment of forest management effects on ECM communities requires study along a longer age trajectory, from post-disturbance to climatic climax stands. Relating ECM community dynamics to forest succession would also be best done over a larger scale so that each developmental stage was represented in proportion to its occurrence over a landscape. Additionally, accurate representations of total fungal community structure would incorporate all growth forms of ECM fungi including sporocarps, root tips, and extramatrical hyphae. Technology to perform such detailed studies does exist, and with time this comprehensive approach will become more feasible and cost efficient.

Below-ground fungal population genetics is a critical field of study to understanding why certain ECM fungi dominate in certain forest stages, and this field is becoming more accessible with application of microsatellite techniques. Microsatellites will also be useful to determine whether Douglas-fir and paper birch can be linked by a common ECM individual. Thus far, fungal individuals have been identified mainly from sporocarps, but their ephemeral nature and mysterious fruiting habits preclude rigorous spatial analysis of fungal distributions.

It was surprising and interesting that host-specific ECM fungi were dominant in young stands. Perhaps a study comparing communities of young stands in which various proportions of either host tree were removed would be informative. If dominance by host-specific fungi was affected by interspecific tree competition, then the relative abundance of host-specific fungi would tend to decrease upon removal of one tree species. However, such a decrease could also result from changes to litter inputs, the soil microclimate, or soil food web, which could also be explored by manipulative field trials. It would also be helpful to study ECM communities of other conifer and broadleaf species from the same sites to see if their proportions of host-specific fungi were similar.

The effect of site environment on ECM parameters remains poorly understood. Non-ECM vascular and nonvascular plants, soil chemistry (including more detailed analysis of organic compounds), and soil microbial communities and their physiology may all affect ECM communities. As mentioned above, it is likely I did not sample soil nutrients at a small enough spatial scale to see relationships with ECM variables. Developing these relationships from more intensive sampling might provide a basis for functional studies on specific ECM taxa. More intensive sampling may also reveal tighter relationships between occurrence of non-ECM plants and ECM fungi.

The soil environment is one of the most complex parts of forest ecosystems. Ectomycorrhizal ecology is still largely focused on identifying species and describing fungal habitat parameters, such as forest stand age and nutrient availability. However, this information is rapidly accumulating, as is understanding of mycorrhizal functions. With more advances in these areas, forest management may be improved so that it sustains a healthy below-ground environment.

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Appendix A

Descriptions and photographs of a few commonly encountered ectomycorrhizae follow; these are intended to provide an example of the rigorous morphotyping approach undertaken in this study. Descriptions generally follow format and protocol of Goodman *et al.* (1996). Each description is followed by one or two pages of photos detailing important features, which were taken on dissecting and compound microscopes as detailed in Chapter 2. ECM fungal species found on both hosts showed different system and tip sizes with host (i.e. larger on Douglas-fir), but microscopic features were of similar size on both hosts. While most photos taken on the compound microscope were taken in black and white, photos taken on the dissecting scope were taken in colour. Therefore, some important details will be lost in black and white printed versions.

Lactarius pubescens on paper birch (pg. 1)

DISTINGUISHING FEATURES: smooth tips with a network of laticifers visible on the mantle surface; tips often white to yellowish with purple apices; outer mantle a net prosenchyma surrounded by a gelatinous matrix; mycelial strands common, with an inner core of laticifers; oleiferous cells present in inner mantle which appear similar to laticifers but do not react in sulphovanillin

MORPHOLOGY (Dissection Microscope):

ECTOMYCORRHIZAL SYSTEM:

Shape and dimensions: (Figs. 1 to 3) monopodial pinnate to pyramidal systems 4 (2-8) mm long by 2.5 (1-4) mm wide; tips 1.5 (0.3-3) mm long by 300 (150-500) µm wide

Colour and texture: (Figs. 1 to 4) White to cream to yellow to pinkish to dark purple, smooth, shiny, host not visible through mantle

EMANATING ELEMENTS:

Mycelial Strands: (Figs. 2 to 4 and 10 to 11) common, white, round in cross section, rarely branched, often running along non-ectomycorrhizal portions of birch roots to which ectomycorrhizal systems are attached; attachments are restricted points at bases of systems

Hyphae: none

Lactarius pubescens on paper birch (pg. 2)

ANATOMY (Compound Microscope):

MANTLE IN PLAN VIEW: thick mantle, Hartig net present

Outer Layer: a net prosenchyma (Figs. 5 to 7) with gelatinous matrix; cells 35 (10-50) µm long by 3 (2-5) µm wide, smooth, hyaline; laticifers common, 5 (3-7) µm wide; junctions common, 90-120°; anastomoses common, H-shaped

Inner Layer: a net synenchyma (Figs. 8 to 9); cells 20 (10-45) µm long by 3 (1-5) µm wide; contact anastomoses common; junctions rare, variable; oleiferous cells common in some samples but absent in others, 4 (2-6) µm wide

MYCELIAL STRANDS IN PLAN VIEW: differentiated (Figs. 10 to11); outer hyphae thick walled, 40 (10-80) µm long by 2.5 (1-3.5) µm wide; inner hyphae are laticifers, 4 (3-5.5) µm wide, length not determined; no junctions or anastomoses seen in either layer

EMANATING HYPHAE: none seen

CYSTIDIA: none seen

OTHER FEATURES:

SCLEROTIA AND MICROSCLEROTIA: none seen

CHLAMYDOSPORES: none seen

AUTOFLOURESCENCE OF WHOLE TIPS: not tested

CHEMICAL REACTIONS: laticifers dark blue to black in sulphovanillin; no reaction to 15% KOH or Melzer's; hyphae turn only slightly blue in toluidine blue

DNA: ITS-region DNA sequences run for several samples

ADDITIONAL CHARACTERS: none observed

Lactarius pubescens on paper birch (pg. 3)

ADDITIONAL INFORMATION:

COLLECTION AND IDENTIFICATION: collected by B.D. Twieg; identified by B.D. Twieg, initially by comparison with description in Ingleby *et al.* (1990), but the current description differs in that oleiferous cells were found in the inner mantle, and photos are more detailed. Identification was confirmed by DNA sequence comparison to online databases (see Appendix B).

ECOLOGY: found many times in 5-yr-old stands

REFERENCES:

Ingleby K, Mason PA, Last FT, Fleming LV. 1990. *Identification of ectomycorrhizae. ITE Research Pulication No. 5.* London: Her Majesty's Stationery Office.

Latarius pubescens
on paper birch (pg. 4)

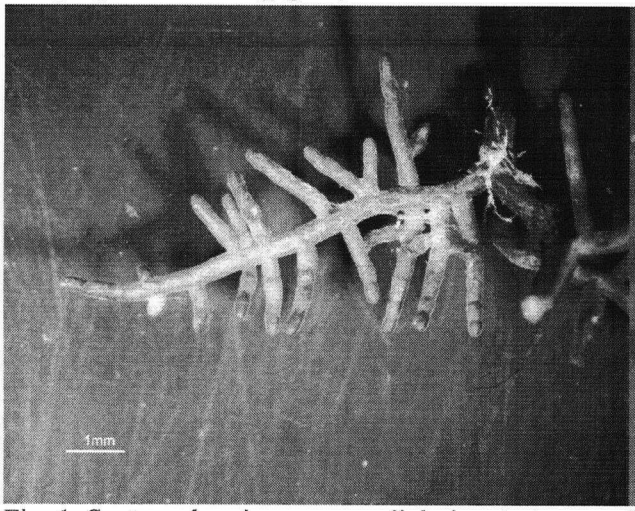

Fig. 1 System showing monopodial pinnate to pyramidal branching and yellow to purple color range

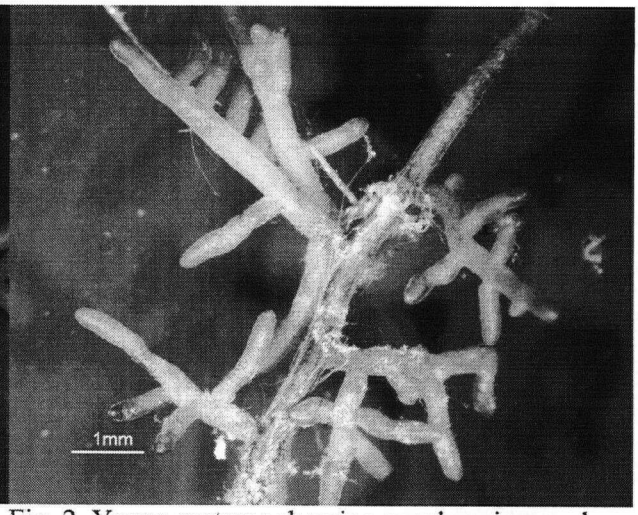

Fig. 2 Young systems showing purple apices and mycelial strands running along main root axis

Fig. 3 Older purple tips and mycelial strand running along main root axis

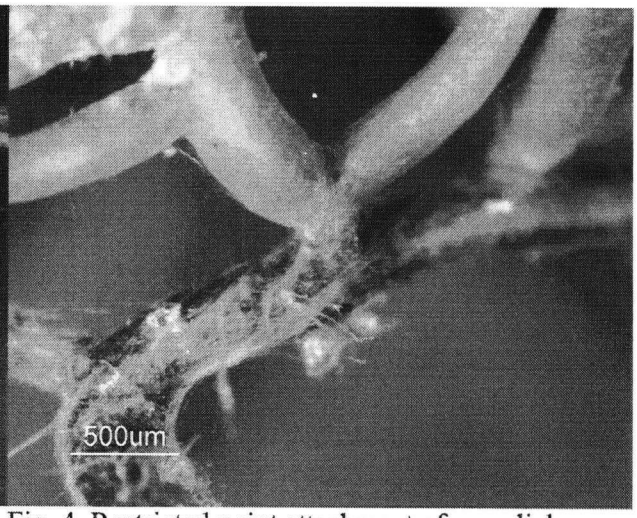

Fig. 4 Restricted point attachment of mycelial strand to base of system

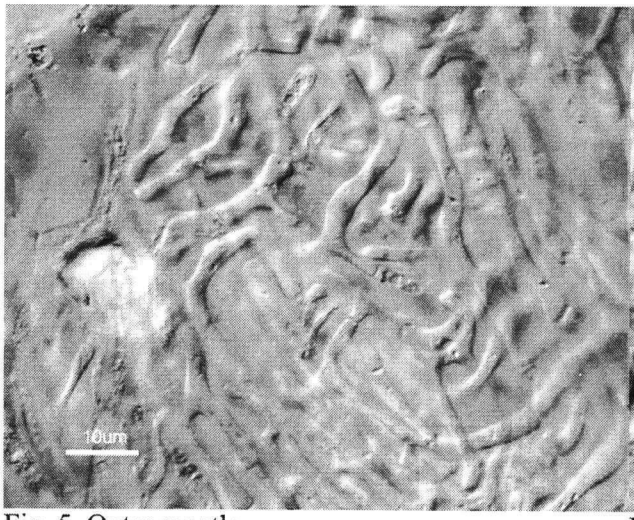

Fig. 5 Outer mantle

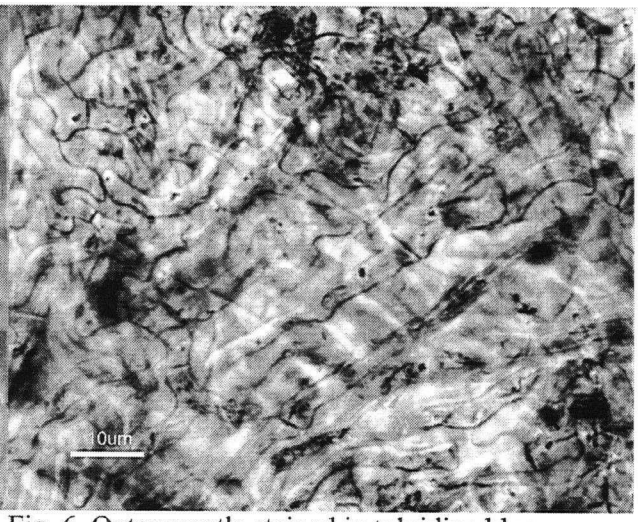

Fig. 6 Outer mantle stained in toluidine blue (black and white photo)

Lactarius pubescens
on paper birch (pg. 5)

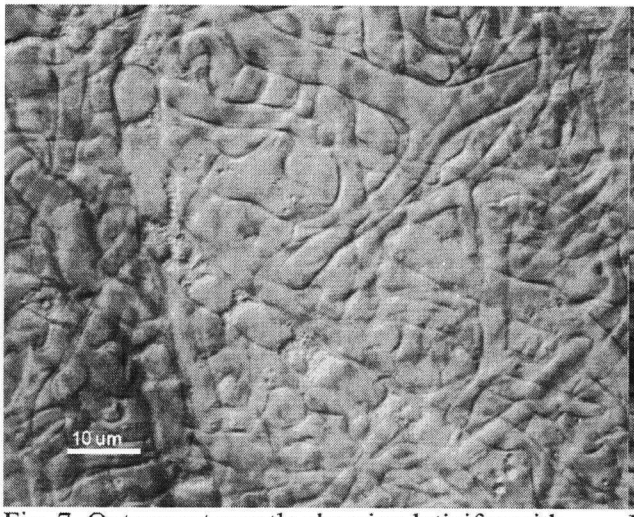

Fig. 7 Outermost mantle showing laticifer with latex and empty laticifer-sized hyphae

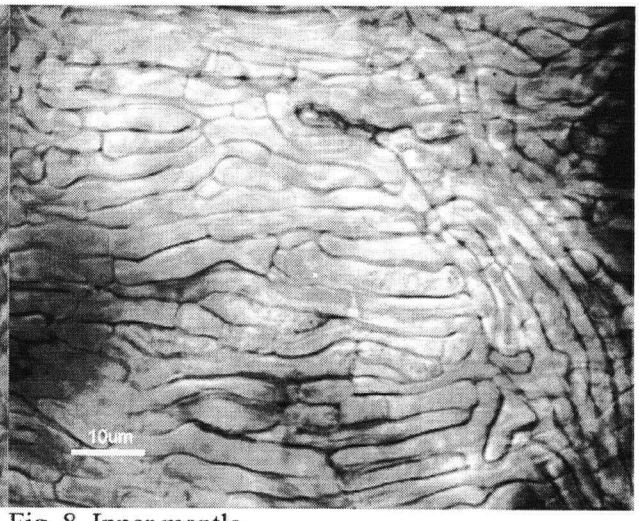

Fig. 8 Inner mantle

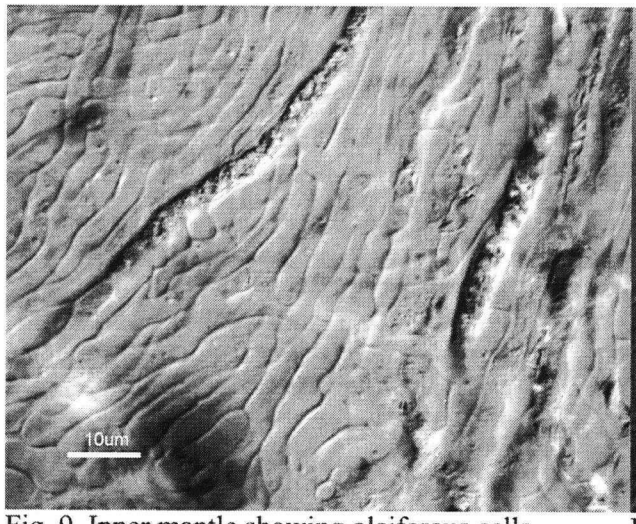

Fig. 9 Inner mantle showing oleiferous cells

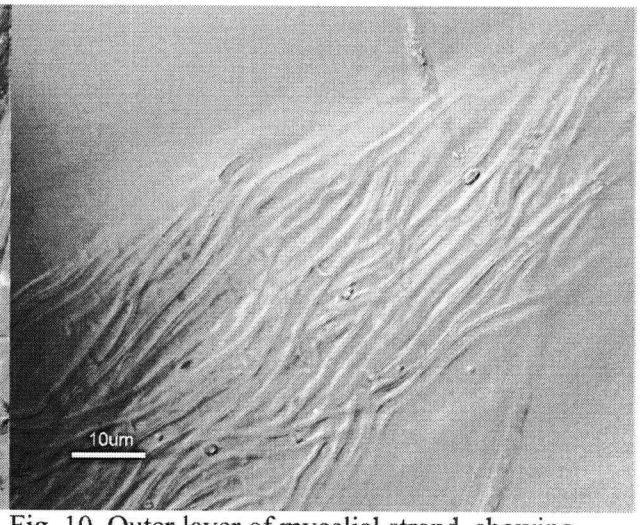

Fig. 10 Outer layer of mycelial strand, showing slightly thickened walls

Fig. 11 Mycelial strand showing laticifers of inner layer and oleiferous cell in sulphovanillin

Lactarius torminosus on paper birch (pg. 1)

DISTINGUISHING FEATURES: large systems with a network of laticifers visible on the mantle surface; systems white with pink-purple patches when young and orange-pink when older; outer mantle a net synenchyma with large intracellular oil bodies; mycelial strands common, with an inner core of laticifers

MORPHOLOGY (Dissection Microscope):

ECTOMYCORRHIZAL SYSTEM:

Shape and dimensions: (Figs. 1 to 3) monopodial pinnate, 7 (2.5-12) mm long by 5 (1.5-7) mm wide; tips 2.5 (1-6) mm long by 350 (250-500) µm wide

Colour and texture: (Figs. 1 to 3) White to pink-purple to pink to orange, smooth, shiny, host not visible through mantle

EMANATING ELEMENTS:

Mycelial Strands: (Figs. 1 and 3) common, white, round in cross section, rarely branched, often running along non-ectomycorrhizal portions of birch roots to which ectomycorrhizal systems are attached; attachments are restricted points at bases of systems (Fig. 3)

Hyphae: none

ANATOMY (Compound Microscope):

MANTLE IN PLAN VIEW: thick mantle, Hartig net present

Outer Layer: a net synenchyma (Fig. 4) with abundant intracellular oil bodies; cells 15 (10-35) µm long by 4 (2.5-7) µm wide, smooth, hyaline, swellings to 10µm around septa; laticifers common (Fig. 5), 6.5 (4.5-8) µm wide; junctions rare, variable; anastomoses not seen

Inner Layer: a net synenchyma (Fig. 6); cells 30 (10-60) µm long by 3.5 (1.5-6.5) µm wide; contact anastomoses common; junctions common, 90-120°; septa common, with rare clamp connections

Lactarius torminosus on paper birch (pg. 2)

MYCELIAL STRANDS IN PLAN VIEW: differentiated (Figs. 7 to 8); outer hyphae thick walled, 70 (15-150) µm long by 3.5 (2-4) µm wide; inner hyphae are laticifers, 6.5 (5-8) µm wide, at least 20 µm long; no junctions or anastomoses seen in either layer

EMANATING HYPHAE: none seen

CYSTIDIA: none seen

OTHER FEATURES:

SCLEROTIA AND MICROSCLEROTIA: none seen

CHLAMYDOSPORES: none seen

AUTOFLOURESCENCE OF WHOLE TIPS: not tested

CHEMICAL REACTIONS: laticifers dark blue to black in sulphovanillin; no reaction to 15% KOH or Melzer's; mantle hyphae turn slightly blue in toluidine blue

DNA: ITS-region DNA sequences run for several samples

ADDITIONAL CHARACTERS: none observed

ADDITIONAL INFORMATION:

COLLECTION AND IDENTIFICATION: collected by B.D. Twieg; identified by B.D. Twieg. Identification was made by DNA sequence comparison to online databases (see Appendix B).

ECOLOGY: found many times in 26- to 100-yr-old stands

Lactarius torminosus
on paper birch (pg. 3)

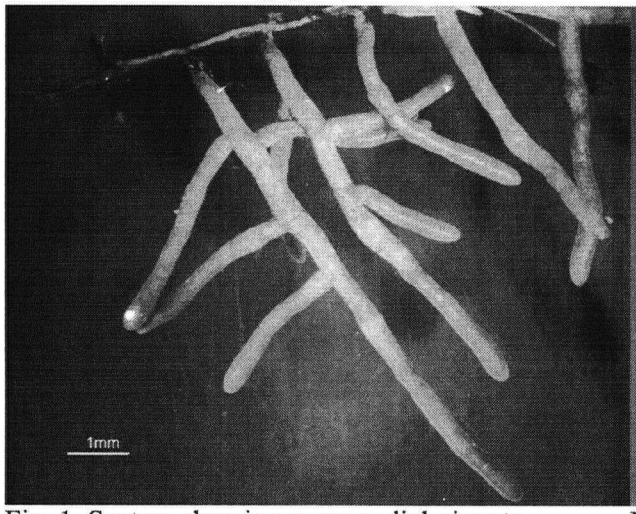

Fig. 1 System showing monopodial pinnate branching and orange to pink color

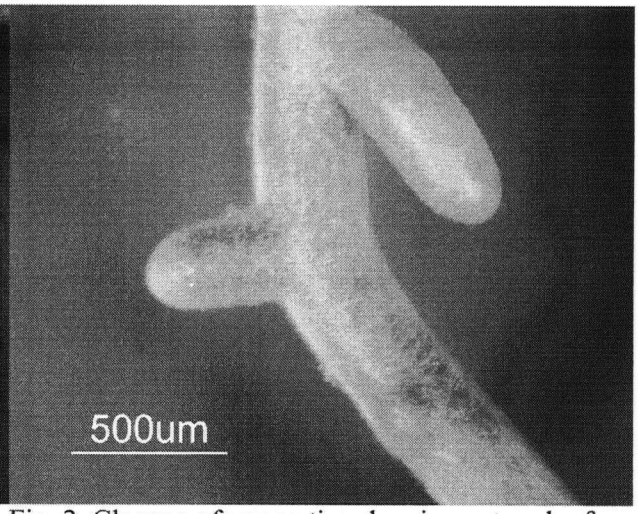

Fig. 2 Closeup of young tips showing network of laticifers on the mantle surface

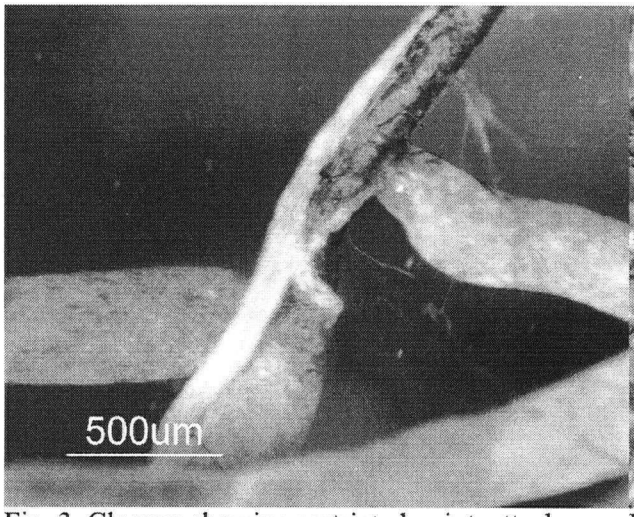

Fig. 3 Closeup showing restricted point attachments of mycelial strand at bases of tip sytems

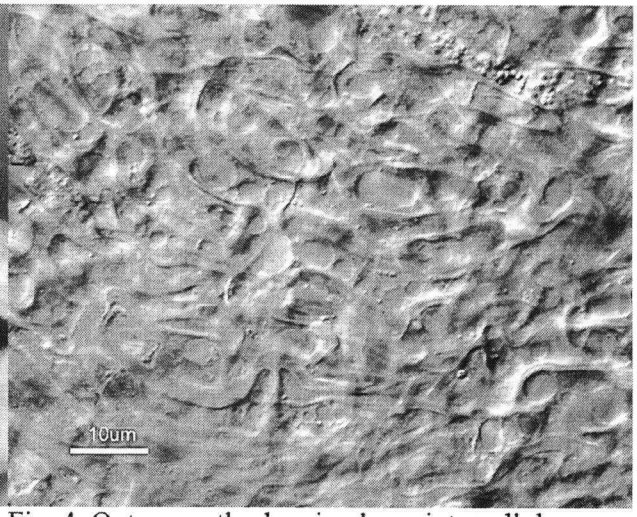

Fig. 4 Outer mantle showing large intracellular oil bodies

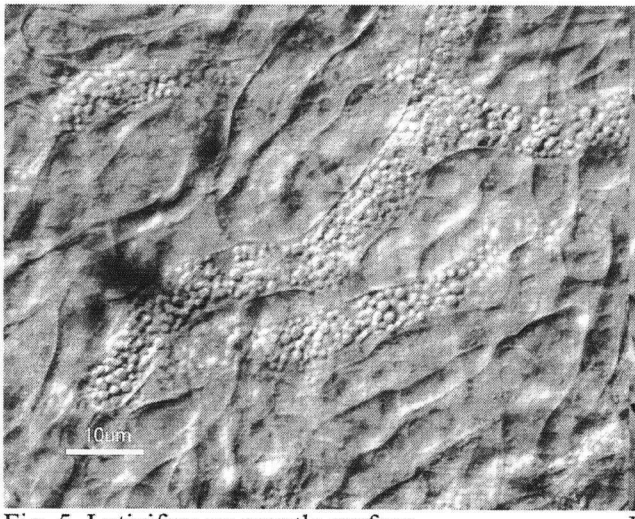

Fig. 5 Laticifers on mantle surface

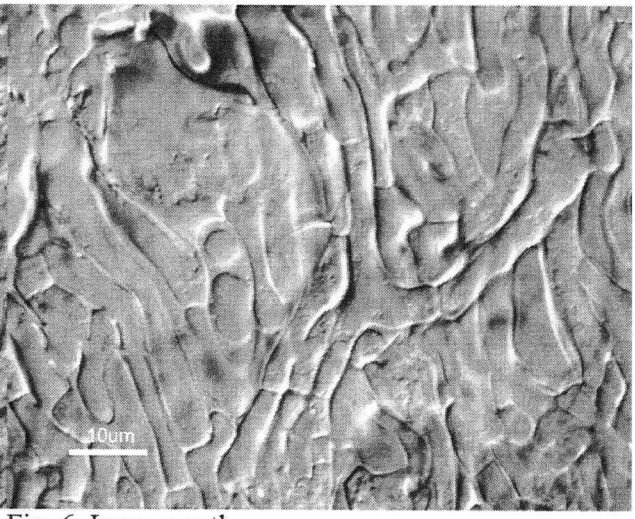

Fig. 6 Inner mantle

Lactarius torminosus
on paper birch (pg. 4)

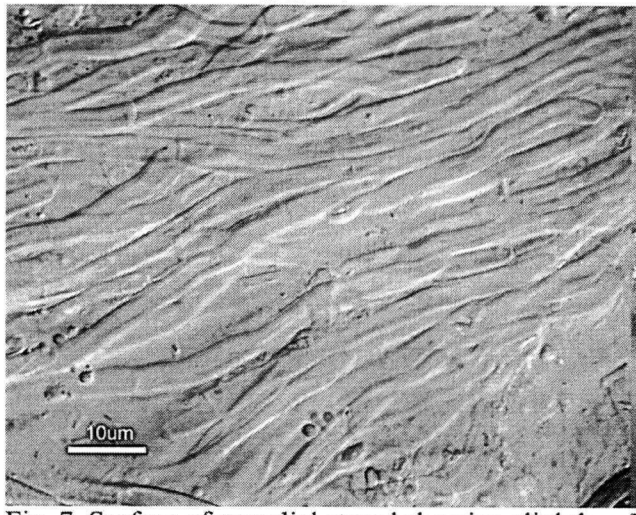

Fig. 7 Surface of mycelial strand showing slightly thickened cell walls

Fig. 8 Inner core of mycelial strand, composed of laticifers

Phallales 1 (*Hysterangium*-like) on Douglas-fir and paper birch (pg. 1)

DISTINGUISHING FEATURES: white systems with abundant white mycelial strands that often have orange to red colour in isolated areas; large, circular ornaments (composed of radiating elements) abundant on mycelial strands and associated hyphae; inflated hyphae common in outer mantle

MORPHOLOGY (Dissection Microscope):

ECTOMYCORRHIZAL SYSTEM:

Shape and dimensions: (Figs. 1 to 5) monopodial pinnate, Douglas-fir systems 7 (4-10) mm long by 3 (1.5-5) mm wide, tips 1 (0.4-5) mm long by 500 (300-700) µm wide; paper birch systems 5 (2-7) mm long by 2.5 (1-4.5) mm wide, tips 0.6 (0.3-1.5) mm long by 350 (250-500) µm wide

Colour and texture: (Figs. 1 to 5) white felty mantle with patches of host tissue sometimes visible; refractive patches sometimes present

EMANATING ELEMENTS:

Mycelial Strands: (Figs. 1 to 5) common, white, flattened in cross section, frequently branched; flat angle attachment

Hyphae: patches of cottony hyphae commonly emanate from strands, and sometimes from mantle

ANATOMY (Compound Microscope):

MANTLE IN PLAN VIEW: variable in thickness, Hartig net present

Outer Layer: a felt prosenchyma (Fig. 8); cells at least 50µm long by 2.5 (1.5-3.5) µm wide, smooth, hyaline, swellings to 6 µm around septa; septa common, unclamped; contact anastomoses common; junctions common, 90-120°

Inner Layer: a net synenchyma(Fig. 9), not regularly distributed; cells 40 (50-80) µm long by 2.5 (1.5-3.5) µm wide; contact anastomoses common; junctions common, variable, septa common, unclamped

MYCELIAL STRANDS IN PLAN VIEW: (Fig. 6) undifferentiated; hyphae 2.5 (2-3.5) µm wide, length undetermined, surface covered in globular ornaments that are 3 (1.5-5) µm wide, protrude up to 2.5 µm, and are composed of radiating elements; septa rare, clamped;

Phallales 1 (*Hysterangium*-like) on Douglas-fir and paper birch (pg. 2)

EMANATING HYPHAE: (Fig. 7) same as hyphae of mycelial strands
CYSTIDIA: none seen

OTHER FEATURES:

SCLEROTIA AND MICROSCLEROTIA: none seen
CHLAMYDOSPORES: none seen

AUTOFLOURESCENCE OF WHOLE TIPS: not tested

CHEMICAL REACTIONS: no reaction to KOH or Melzer's; other chemicals not tested

DNA: ITS region DNA sequences run for several samples

ADDITIONAL CHARACTERS: none observed

ADDITIONAL INFORMATION:

COLLECTION AND IDENTIFICATION: collected by B.D. Twieg; identified by B.D. Twieg. Morphotype matched well to *Hysterangium crassirachis* (Agerer, 1987), but sequence aligned to various *Ramaria* and *Gautieria* species over only short (~150 bp) segments in web searches. Identification was made by DNA sequence comparison to online databases (see Appendix B).

ECOLOGY: found many times in 26- to 65-yr-old stands

REFERENCES:

Agerer R. 1987. *Colour Atlas of Ectomycorrhizae* . Schwäbisch Gmünd, Germany: Einhorn.

Phallales 1 (*Hysterangium*-like) on Douglas-fir and paper birch (pg. 3)

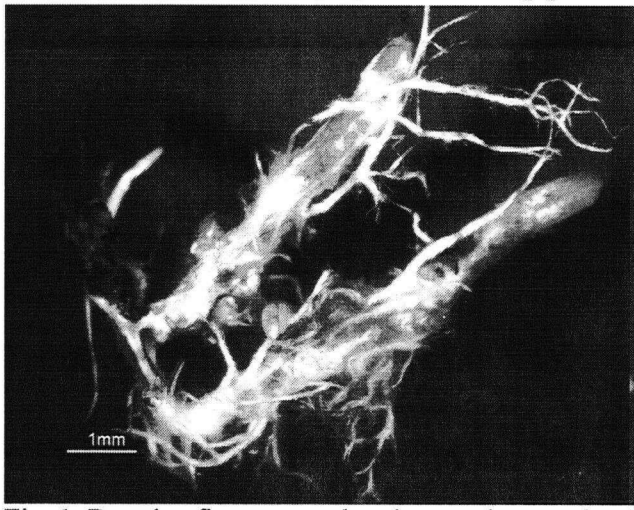

Fig. 1 Douglas-fir systems showing patchy mantle and reddish hue in some mycelial strands

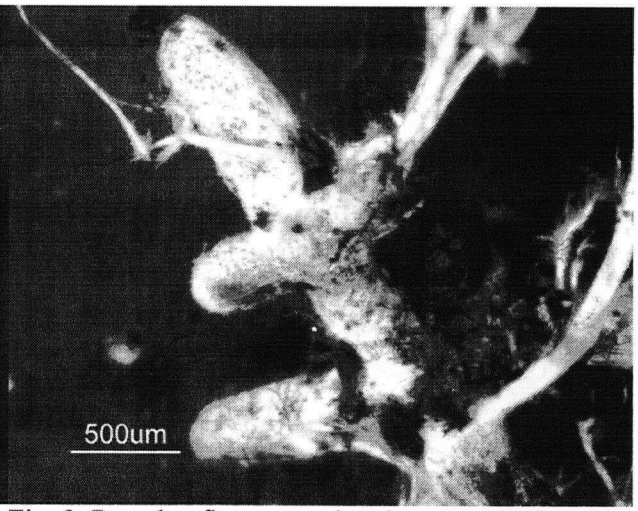

Fig. 2 Douglas-fir system showing monopodial pinnate branching

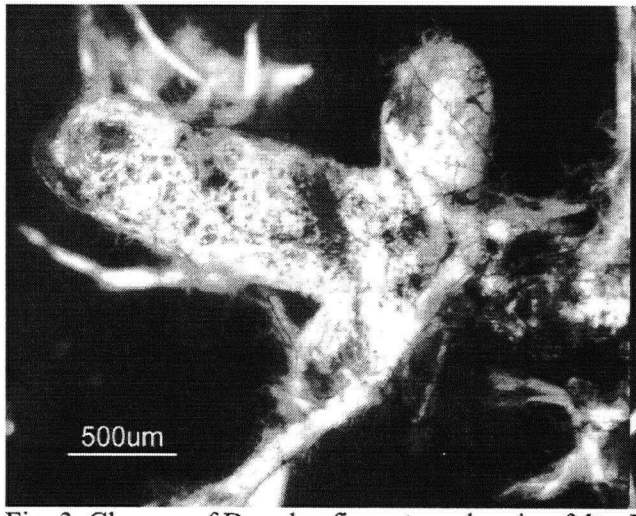

Fig. 3 Closeup of Douglas-fir system showing felty mantle and reddish color on mycelial strands

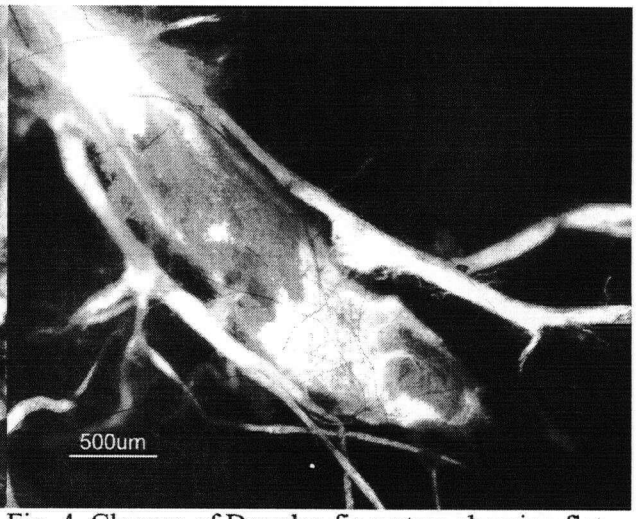

Fig. 4 Closeup of Douglas-fir system showing flat angle mycelial strand attachment

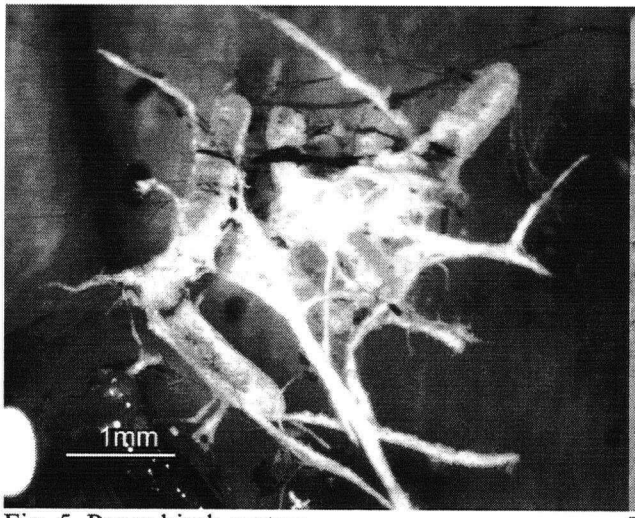

Fig. 5 Paper birch system

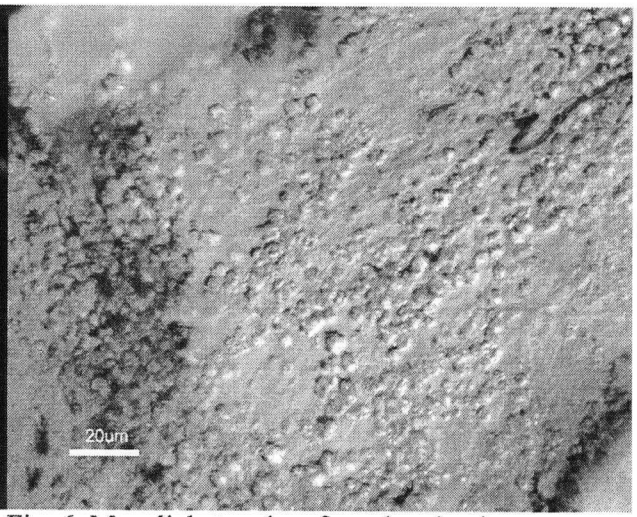

Fig. 6 Mycelial strand surface showing heavy crystal encrustation (from paper birch tip)

Phallales 1 (*Hysterangium*-like) on Douglas-fir and paper birch (pg. 4)

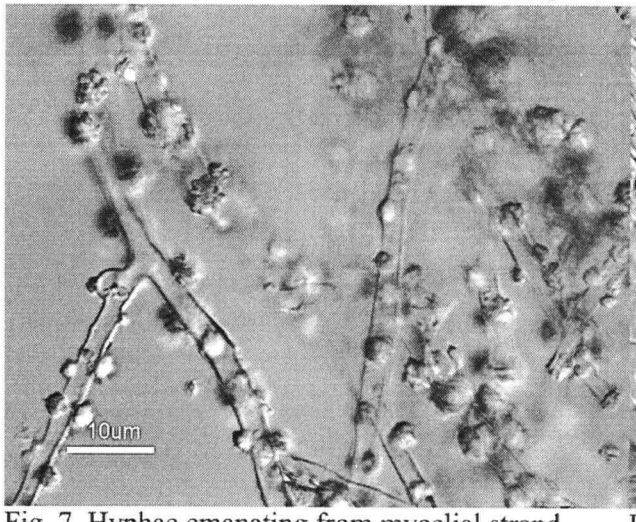

Fig. 7 Hyphae emanating from mycelial strand showing clamp connection and ornamentation

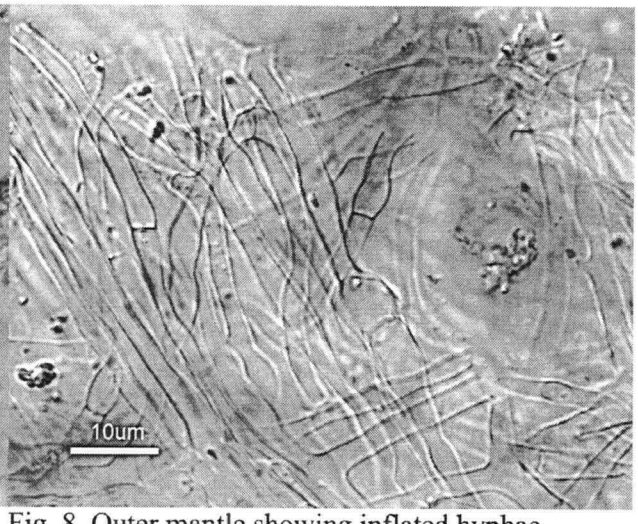

Fig. 8 Outer mantle showing inflated hyphae

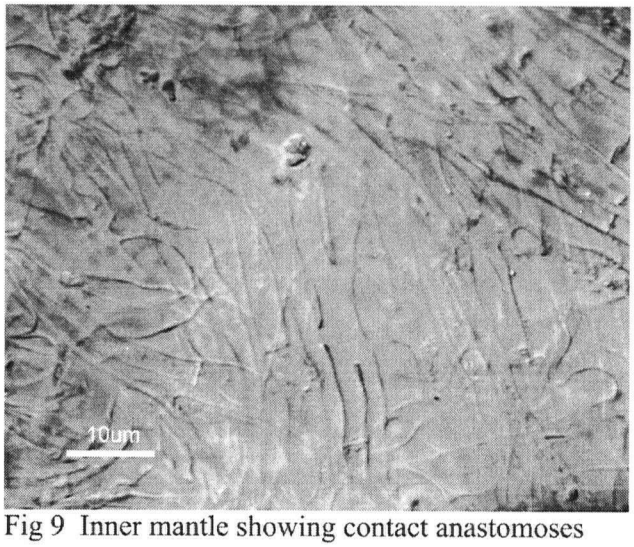

Fig 9 Inner mantle showing contact anastomoses

Russula aeruginea on Douglas-fir and paper birch (pg. 1)

DISTINGUISHING FEATURES: tips have a short-spiny surface over at least part of tips, caused by long (average 55 µm), bristle-like cystidia with inflated bases; other parts of surface have a warty or frosted appearance; outer mantle is an irregular non-interlocking synenchyma composed of ovoid cells,

MORPHOLOGY (Dissection Microscope):

ECTOMYCORRHIZAL SYSTEM:

Shape and dimensions: (Figs. 1 to 3) monopodial pinnate, Douglas-fir systems 6 (3-8) mm long by 2.5 (1.5-5) mm wide, tips 1.5 (0.25-2.5) mm long by 400 (200-600) µm wide; paper birch systems 4 (2-7) mm long by 2 (1-4.5) mm wide, tips 1 (0.25-2) mm long by 300 (200-500) µm wide on paper birch

Colour and texture: (Figs. 1 to 4) light brown to pink to purple with white reflective patches; short spiny in parts and finely warty or frosted-looking in others

EMANATING ELEMENTS:

Mycelial Strands: none seen

Hyphae: none seen

ANATOMY (Compound Microscope):

MANTLE IN PLAN VIEW: variable in thickness, Hartig net present

Outer Layer: (Fig. 5 and 6) a non-interlocking irregular synenchyma; cells smooth, with granular contents, ovoid, 8 (5-13) µm long by 5 (3-7) µm wide; no septa within ovoid cells; septa at bases of ovoid cells unclamped; clumps of extra layers of these cells likely give tips their warty or frosted appearance

Inner Layer: a net prosenchyma, cells smooth, hyaline, 30 (10-50) µm long by 3 (2-4) µm wide; anastomoses rare, H-shaped; junctions common, 90-120°; septa common, unclamped

MYCELIAL STRANDS IN PLAN VIEW: none seen

EMANATING HYPHAE: none seen

CYSTIDIA: abundant; tapering to a point but not thick-walled; hyaline; no contents; 55 (30-70) µm long by 2.5 (1-4) µm wide (5-6 µm wide at base)

OTHER FEATURES:

SCLEROTIA AND MICROSCLEROTIA: none seen
CHLAMYDOSPORES: none seen

AUTOFLOURESCENCE OF WHOLE TIPS: not tested

CHEMICAL REACTIONS: no reaction to KOH, Melzer's, or sulphovanillin; cystidia strongly purple and outer mantle faintly blue in toluidine blue

DNA: ITS region DNA sequences run for several samples

ADDITIONAL CHARACTERS: none observed

ADDITIONAL INFORMATION:

COLLECTION AND IDENTIFICATION: collected by B.D. Twieg; identified by B.D. Twieg. Morphotype matched to Kranabetter & Friesen's (2004) description of *R. aeruginea* on pine. Identification was made by DNA sequence comparison to online databases (see Appendix B).

ECOLOGY: found several times in 26- to 100-yr-old stands

REFERENCES:

Kranabetter M, Friesen J. 2004. *Morphotype Descriptions of Common Ectomycorrhizal Fungi.* May 2006.

Russula aeruginea
on Douglas-fir and paper birch (pg. 3)

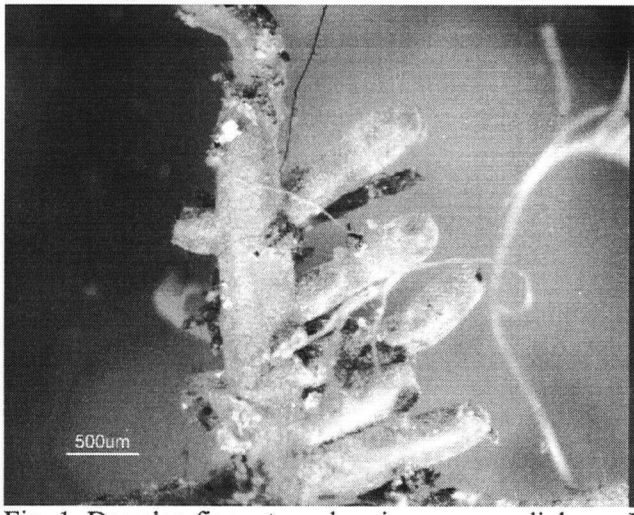

Fig. 1 Douglas-fir system showing monopodial pinnate branching; mycelial strand does not belong

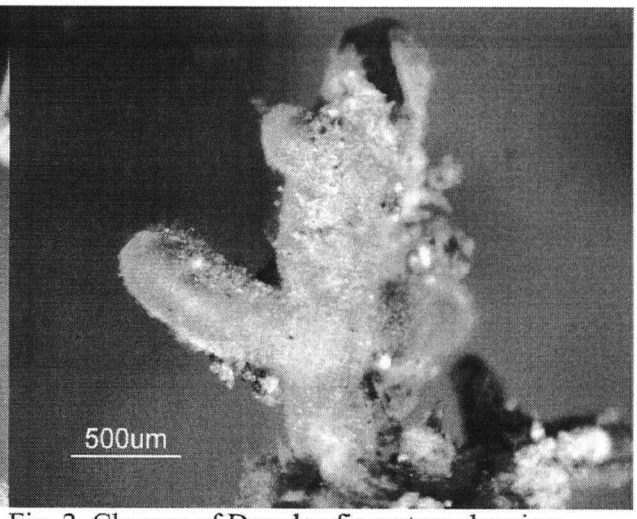

Fig. 2 Closeup of Douglas-fir system showing frosted and short-spiny textures of surface

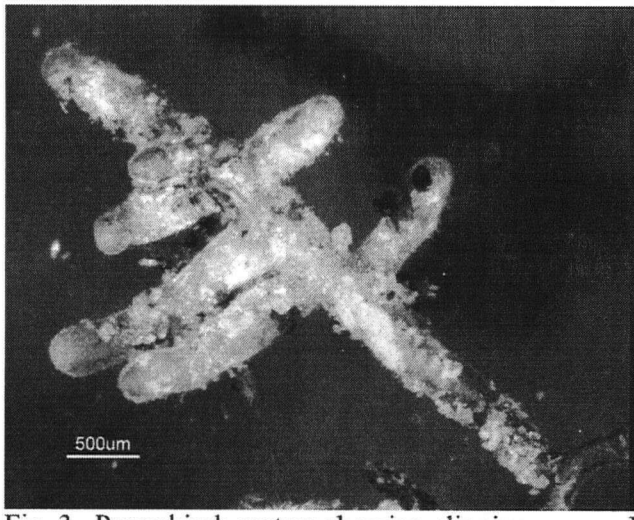

Fig. 3 Paper birch system showing clinging mineral soil and reflective patches

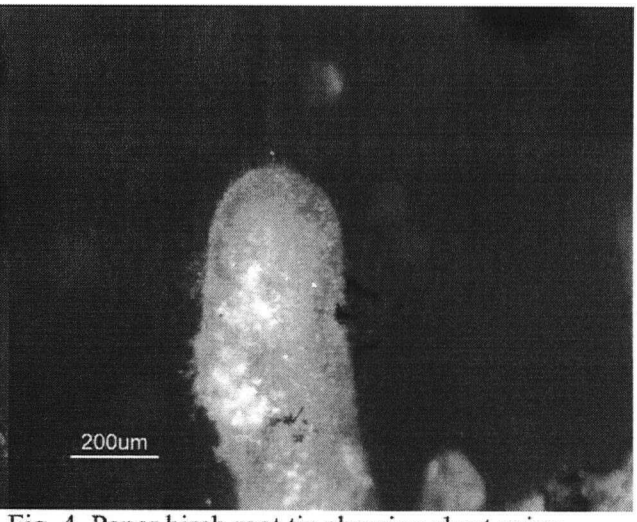

Fig. 4 Paper birch root tip showing short spiny surface texture

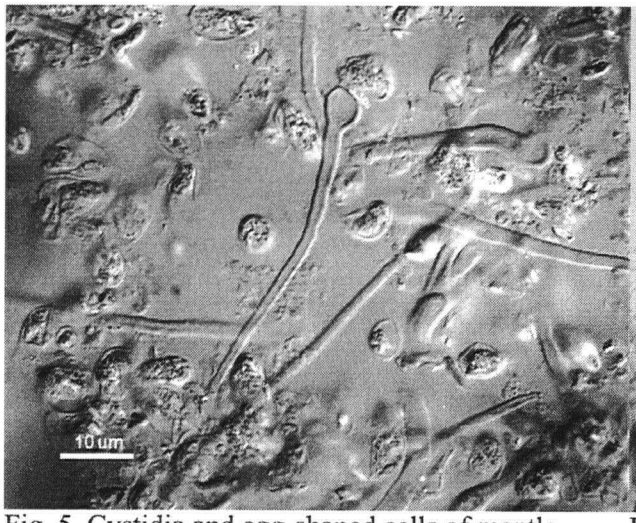

Fig. 5 Cystidia and egg-shaped cells of mantle surface; from Douglas-fir root tip

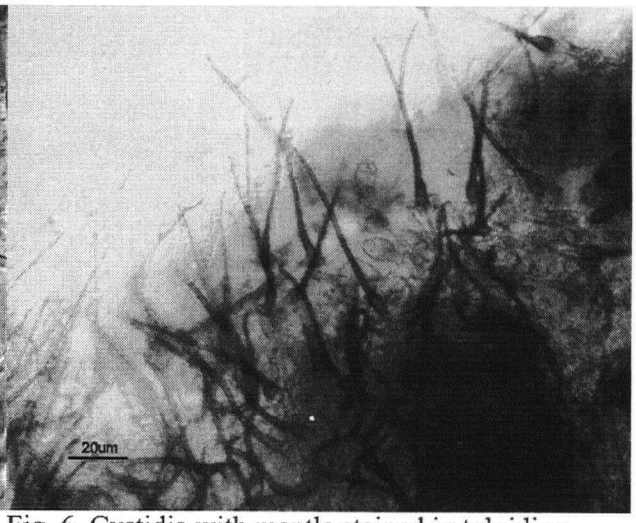

Fig. 6 Cystidia with mantle stained in toluidine blue; from paper birch root tip

Russula brevipes on Douglas-fir (pg. 1)

DISTINGUISHING FEATURES: large systems with a velvety appearance due to abundant and regularly distributed, long cystidia

MORPHOLOGY (Dissection Microscope):

ECTOMYCORRHIZAL SYSTEM:

Shape and dimensions: (Figs. 1 to 3) monopodial pinnate, 9 (5-20) mm long by 7 (4-10) mm wide; tips 4 (1-7) mm long by 600 (400-750) µm wide

Colour and texture: (Figs. 2 and 3) light pink to brown to copper with purple patches; texture velvety

EMANATING ELEMENTS:

Mycelial Strands: none seen

Hyphae: none seen

ANATOMY (Compound Microscope):

MANTLE IN PLAN VIEW: medium-thick, Hartig net present

Outer Layer: (Fig. 5) a net prosenchyma

Inner Layer: a net prosenchyma, cells smooth, hyaline, 15 (10-30) µm long by 2.5 (2-3) µm wide; anastomoses common, contact-type to H-shaped; junctions common, 120°; septa common, unclamped; hyphae often swollen to up to 8 µm around septa

MYCELIAL STRANDS IN PLAN VIEW: none seen

EMANATING HYPHAE: none seen

CYSTIDIA: bottle-shaped, 40 (20-55) µm long by 3 (2-4) µm wide (6-7 µm wide at base), with refractive oily contents; one or two apical buds rarely present

Russula brevipes on Douglas-fir (pg. 2)

OTHER FEATURES:

SCLEROTIA AND MICROSCLEROTIA: none seen
CHLAMYDOSPORES: none seen

AUTOFLOURESCENCE OF WHOLE TIPS: not tested

CHEMICAL REACTIONS: no reaction to KOH, Melzer's, or sulphovanillin; cystidia and mantle pink to light purple in toluidine blue

DNA: ITS region DNA sequences run for several samples

ADDITIONAL CHARACTERS: none observed

ADDITIONAL INFORMATION:

COLLECTION AND IDENTIFICATION: collected by B.D. Twieg; identified by B.D. Twieg. Identification was made by DNA sequence comparison to online databases (see Appendix B).

ECOLOGY: found several times in 26- to 100-yr-old stands

Russula brevipes
on Douglas-fir (pg. 3)

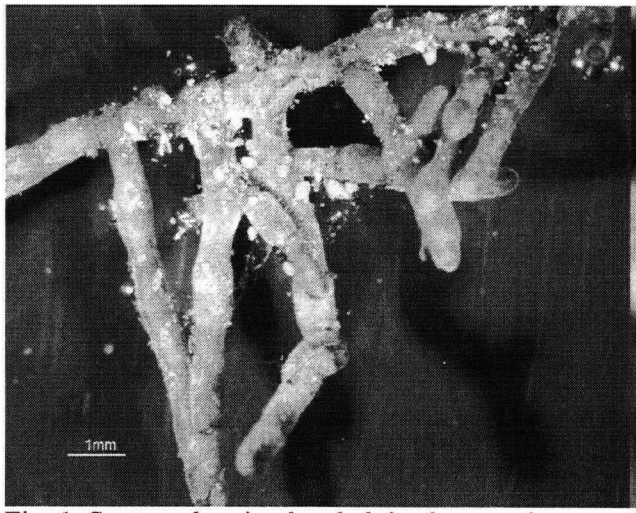

Fig. 1 System showing beaded tip shape and monopodial pinnate branching.

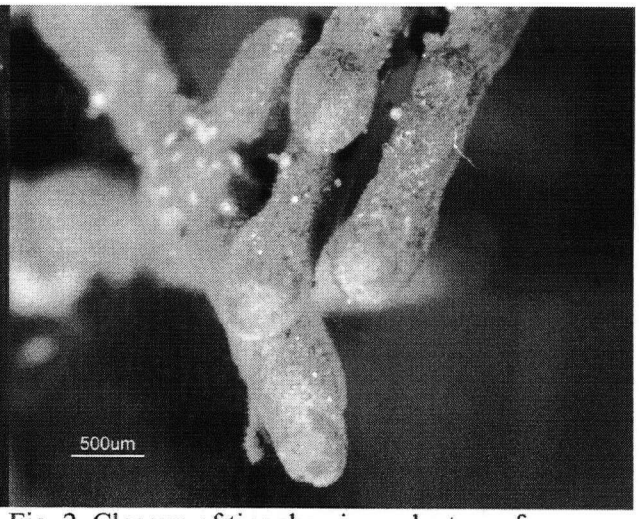

Fig. 2 Closeup of tips showing velvety surface texture and inflated apices.

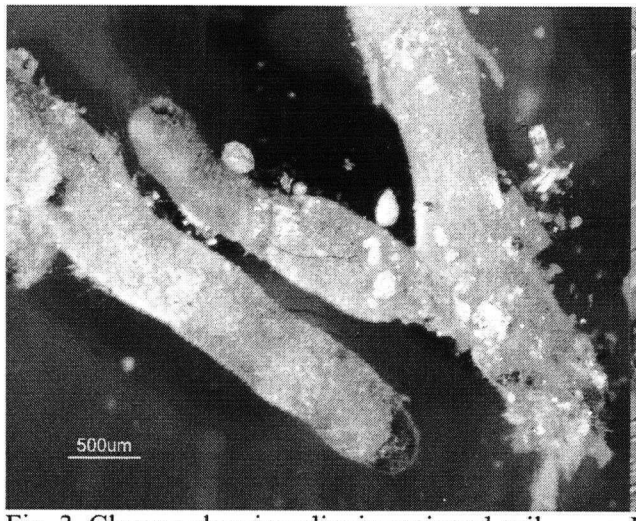

Fig. 3 Closeup showing clinging mineral soil

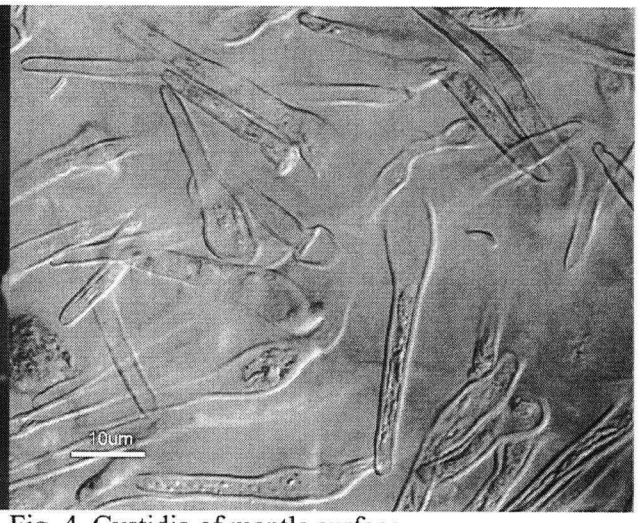

Fig. 4 Cystidia of mantle surface

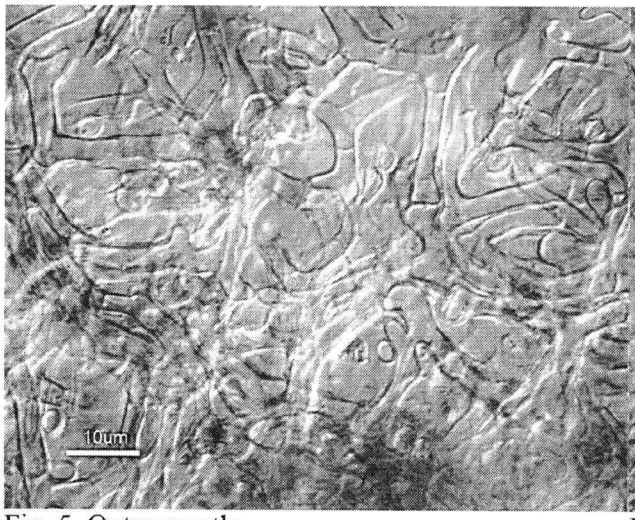

Fig. 5 Outer mantle

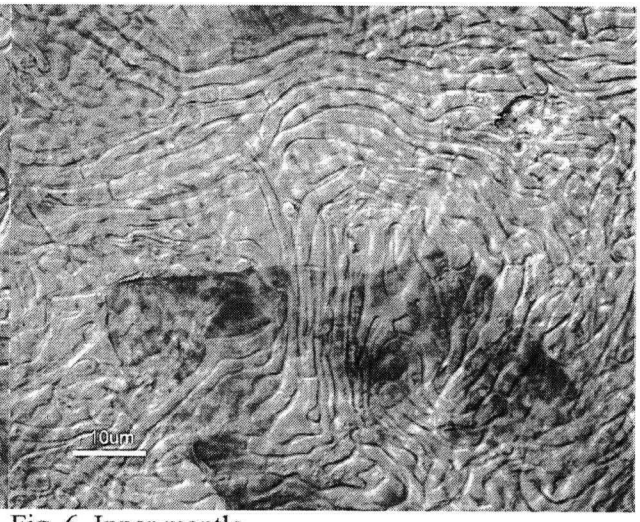

Fig. 6 Inner mantle

Tomentella 10 on paper birch and Douglas-fir (pg. 1)

DISTINGUISHING FEATURES: irregularly branched systems with white felty patches and brown smooth patches; thin brown strands usually present; outer mantle a felt prosenchyma ornamented with irregularly-shaped verrucae; short, pointed cystidia present, also covered in verrucae

MORPHOLOGY (Dissection Microscope):

ECTOMYCORRHIZAL SYSTEM:

Shape and dimensions: (Figs. 1 to 3) irregularly branched, paper birch systems 3 (2-6) mm long by 1.5 (1-4) mm wide, tips 1.5 (1-4) mm long by 300 (250-500) µm wide; Douglas-fir systems 6 (4-10) mm long by 5 (1-7) mm wide, tips 5 (3-8) mm long by 500 (400-700) µm wide

Colour and texture: (Figs. 2 to 5) white and brown; white patches felty, matte to reflective; brown patches smooth; matte to shiny, with localized dark-blue to black patches

EMANATING ELEMENTS:

Mycelial Strands: common, brown, round in cross section, commonly branched, wiry-looking, flat-angle attachment to tips

Hyphae: none seen

ANATOMY (Compound Microscope):

MANTLE IN PLAN VIEW: medium-thick, Hartig net present

Outer Layer: (Fig. 6) a felt prosenchyma; cells at least 8 µm long (max. length not determined) by 2.2 (1.5-3.5) µm wide, sometimes inflated at middle, heavily verrucose; anastomoses common, H-shaped; junctions common, sometimes inflated, 90-120°; septa common, unclamped

Inner Layer: (Fig. 7) a net synenchyma, cells smooth, mostly hyaline, 50 (15-70) µm long by 3 (1.5-3.5) µm wide; patches of dark-blue to black granular contents present; anastomoses common, contact to H-shaped; junctions common, inflated, angle variable; septa common, unclamped

Tomentella 10 on paper birch and Douglas-fir (pg. 2)

MYCELIAL STRANDS IN PLAN VIEW: (Fig. 8) differentiated-random hyphae; 35 (20-80) µm wide; narrow hyphae smooth, brown, 2.5 (1.5-3) µm wide, septa rare, unclamped; wide hyphae heavily verrucose, hyaline, 6 (4-7) µm wide; septa rare, unclamped

EMANATING HYPHAE: none seen

CYSTIDIA: (Fig. 6) sparsely distributed, pointed, verrucose, 10 (7-12) µm long by 3(2.5-4.5) µm long

OTHER FEATURES:

SCLEROTIA AND MICROSCLEROTIA: none seen

CHLAMYDOSPORES: none seen

AUTOFLOURESCENCE OF WHOLE TIPS: not tested

CHEMICAL REACTIONS: no reaction to Melzer's, or sulphovanillin; dark granular contents of inner mantle slightly greenish in KOH

DNA: ITS region DNA sequences run for several samples

ADDITIONAL CHARACTERS: none observed

ADDITIONAL INFORMATION:

COLLECTION AND IDENTIFICATION: collected by B.D. Twieg; identified by B.D. Twieg. Identification was made to genus by comparison of morphological features to manuals (Goodman *et al.*, 1996; Agerer, 1987), and by DNA sequence comparison to online databases (see Appendix B).

ECOLOGY: found infrequently in 5-, 26, and 100-yr-old stands; several occurrences of this morphotype on paper birch were found, but it was found only once on Douglas-fir

Tomentella 10 on paper birch and Douglas-fir (pg. 3)

REFERENCES:

Agerer R. 1987. *Colour Atlas of Ectomycorrhizae* . Schwäbisch Gmünd, Germany: Einhorn.

Goodman DM, Durall DM, Trofymow JA. 1996. *A manual of concise descriptions of North American ectomycorrhizae*. Sydney, B.C.: Mycologue.

Tomentella 10
on paper birch and Douglas-fir (pg. 4)

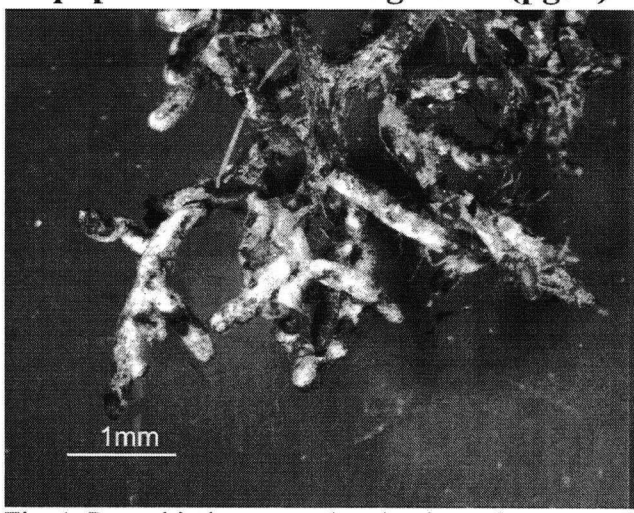

Fig. 1 Paper birch system showing irregular branching and clinging forest floor material

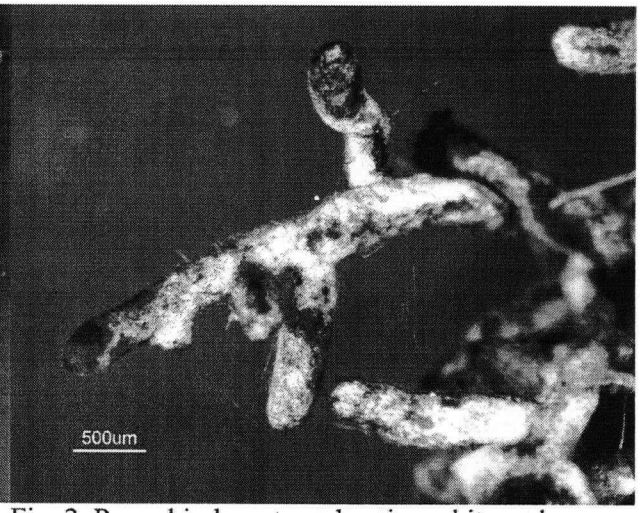

Fig. 2 Paper birch system showing white and brown tip colors

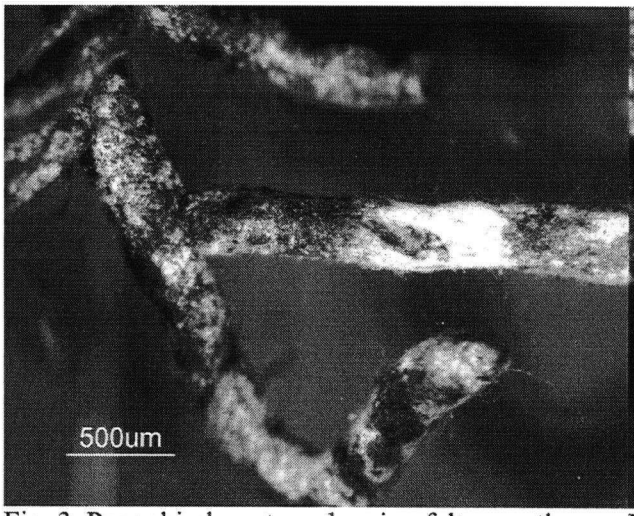

Fig. 3 Paper birch system showing felty mantle patches

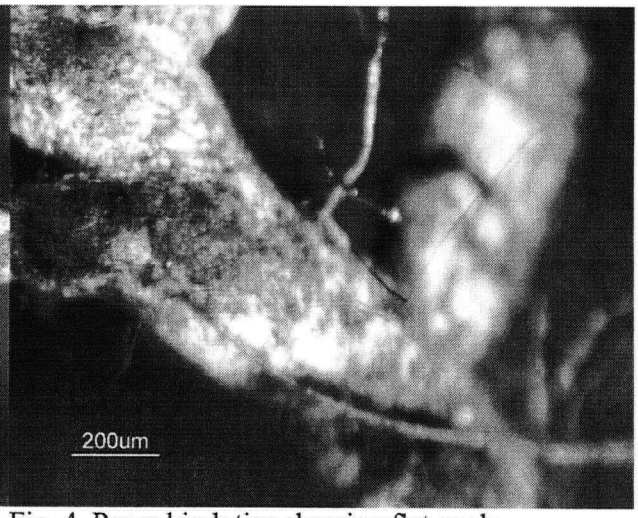

Fig. 4 Paper birch tips showing flat angle mycelial strand attachment

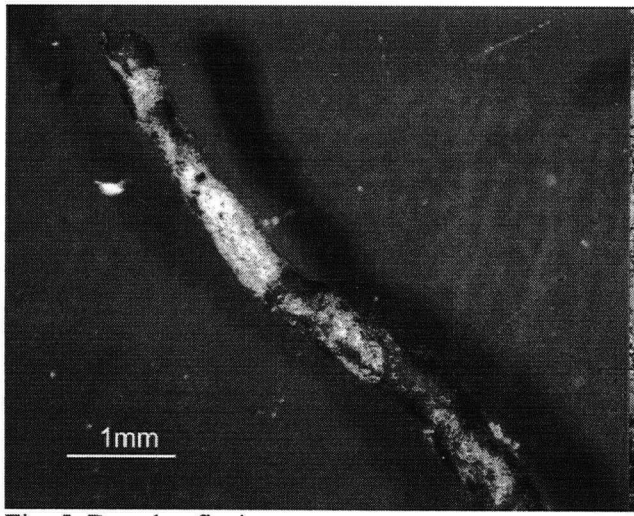

Fig. 5 Douglas-fir tip

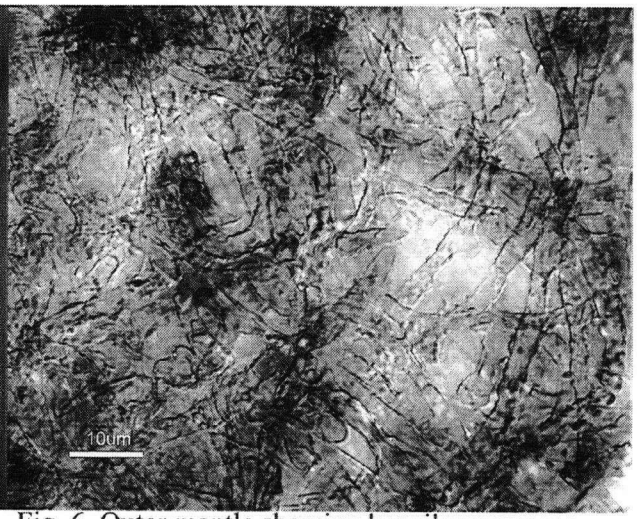

Fig. 6 Outer mantle showing heavily verrucose hyphae and pointed cystidium (center)

***Tomentella* 10**
on paper birch (pg. 5)

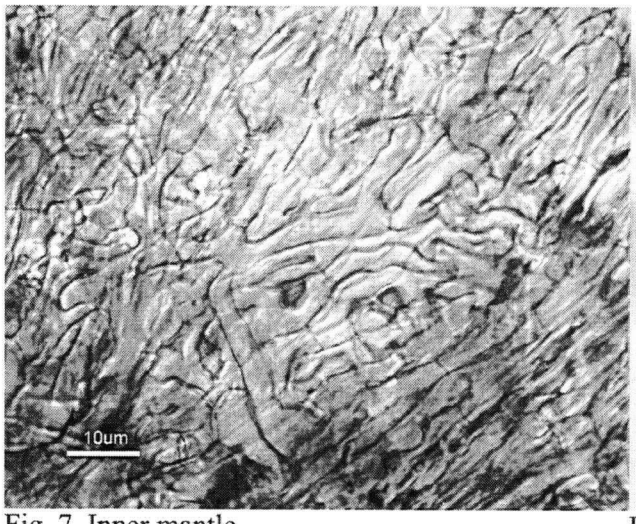

Fig. 7 Inner mantle

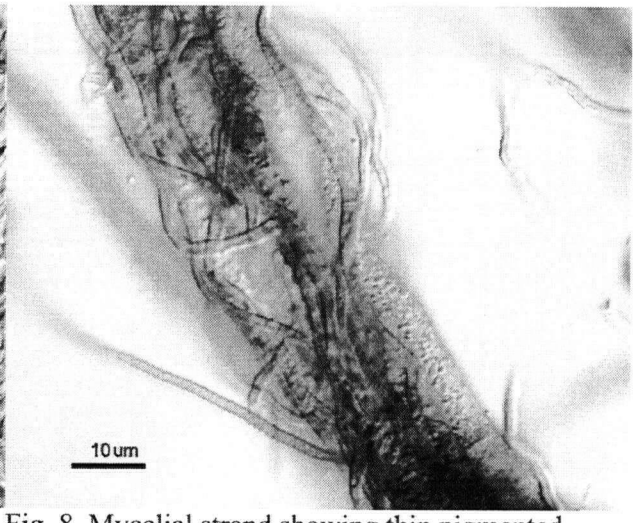

Fig. 8 Mycelial strand showing thin pigmented hyphae and wide verrucose hyphae

Tuber 1 on Douglas-fir (pg. 1)

DISTINGUISHING FEATURES: gold to orange-brown to brown systems that are often short-spiny due to characteristic long, thick-walled, awl-shaped cystidia; tips sometimes appear finely grainy due to additional bundles of short cystidia that are sometimes branched

MORPHOLOGY (Dissection Microscope):

ECTOMYCORRHIZAL SYSTEM:

Shape and dimensions: (Figs. 1 to 2) simple to monopodial pinnate, but sometimes corraloid, 6 (3-10) mm long by 6 (2-8) mm wide; tips 3 (0.2-5) mm long by 450 (400-550) µm wide

Colour and texture: (Figs. 1 to 3) gold to orange-brown to brown; smooth to short-spiny to finely grainy; matte to shiny; host tissue not visible

EMANATING ELEMENTS:

Mycelial Strands: none seen

Hyphae: none seen

ANATOMY (Compound Microscope):

MANTLE IN PLAN VIEW: medium-thick, Hartig net present

Outer Layer: (Fig. 5) a net prosenchyma usually only one cell layer thick; cells hyaline, 10 (8-20) µm long by 2 (2-8) µm wide, highly branched with pentagonal or hexagonal spaces often seen between cells; junctions common, sometimes inflated, 120°; septa common, unclamped, often with an obvious pore

Inner Layer: (Fig. 6) an irregular interlocking synenchyma; cells hyaline, 15 (10-20) µm long by 6 (4-12) µm wide, usually smooth but sometimes with a rugose surface; septa, anastomoses, and junctions not seen

MYCELIAL STRANDS IN PLAN VIEW: none seen

EMANATING HYPHAE: none seen

Tuber 1 on Douglas-fir (pg. 2)

CYSTIDIA: (Fig. 4) two types present: one type is awl-shaped, 55 (40-75) µm long by 2.5 (2-2.75) µm wide, walls thick (~1µm); other type is short, often branched and occurring in bundles, 9 (5-12) µm long by 4 (3-5) µm wide, slightly tapered from base upward

OTHER FEATURES:

SCLEROTIA AND MICROSCLEROTIA: none seen
CHLAMYDOSPORES: none seen

AUTOFLOURESCENCE OF WHOLE TIPS: not tested

CHEMICAL REACTIONS: no reaction to KOH, Melzer's, or sulphovanillin

DNA: ITS region DNA sequences run for several samples

ADDITIONAL CHARACTERS: none observed

ADDITIONAL INFORMATION:

COLLECTION AND IDENTIFICATION: collected by B.D. Twieg; identified by B.D. Twieg. Identification was made to genus by comparison of morphological features to manuals (Agerer, 1987), and by DNA sequence comparison to online databases (see Appendix B).

ECOLOGY: found infrequently in all stand types; several occurrences of this species were found on both hosts studied

REFERENCES:

Agerer R. 1987. *Colour Atlas of Ectomycorrhizae* . Schwäbisch Gmünd, Germany: Einhorn.

Tuber 1
on Douglas-fir (pg. 3)

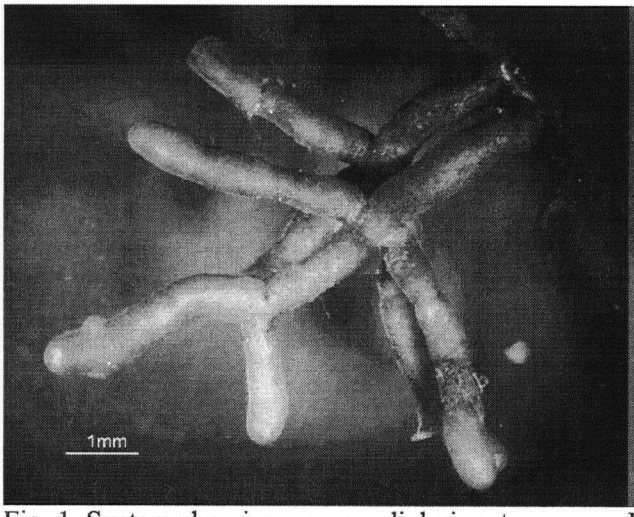

Fig. 1 System showing monopodial pinnate branching

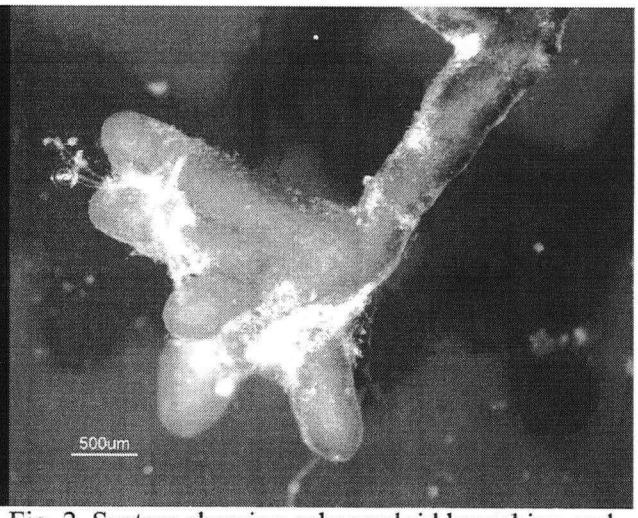

Fig. 2 System showing subcorraloid branching and clinging mineral soil

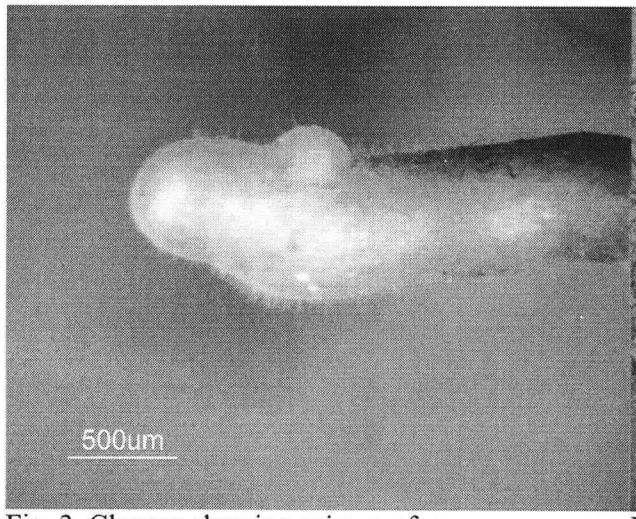

Fig. 3 Closeup showing spiny surface

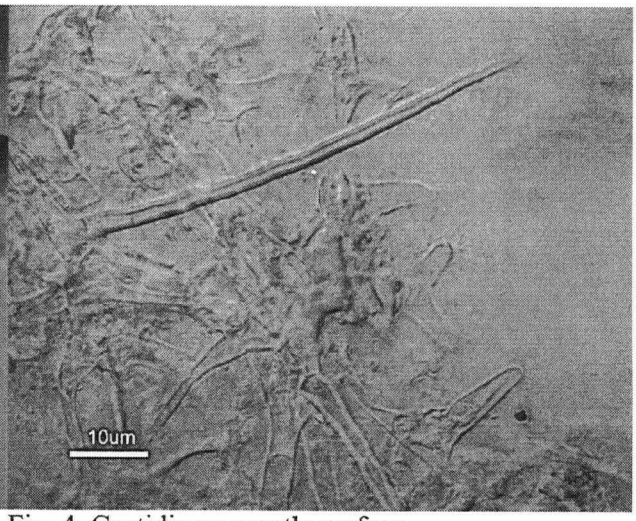

Fig. 4 Cystidia on mantle surface

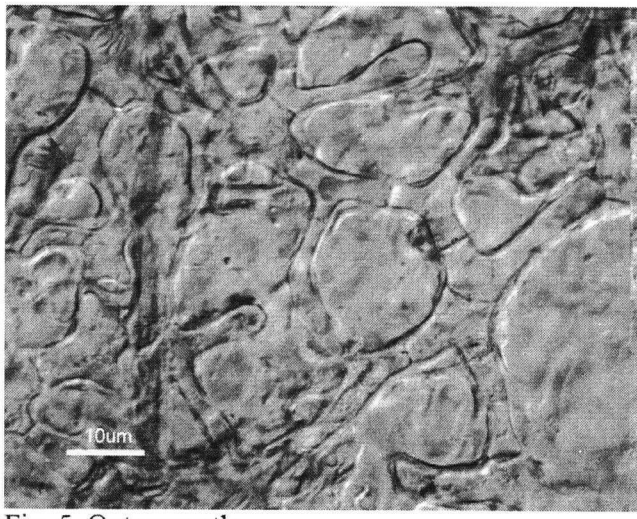

Fig. 5 Outer mantle

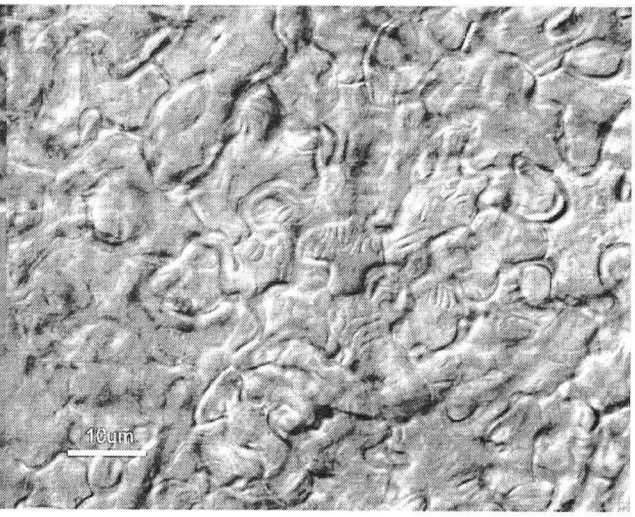

Fig. 6 Inner mantle

Appendix B

This table is a list of ECM taxa and web-based alignment/match information. *s are present in UNITE database accession numbers; this means to insert three zeros to obtain the complete number. Other accession numbers are for NCBI-linked databases. Mean relative abundances are means of four replicate sites for each stand type (age and cc = clearcut, b = burned), and are respective of the host listed in the Host column. Fir = Douglas-fir; Birch = paper birch.

Functional "Species" Name	Closest NCBI or UNITE BLAST Match	Accession Number	Total Base Pairs Aligned[1]	% Sim.[2]	Host	Mean Relative Abundance (%)				
						5 cc	26 b	26 cc	65 b	100 b
Agaricales 1	*Entoloma nitidum*	AY228340	491	88%	Both	0	0	0	0	3.6
Amphinema byssoides	*Amphinema byssoides*	AY838271	623	98%	Both	1.5	3.4	0.4	2.0	2.5
Atheliaceae 1	Uncultured ECM	AF476986	593	99%	Birch	0	0	1.0	0	0
Boletus 1	*Boletus calopus*	AJ889928	684	97%	Birch	0	1.0	0	0	0
Cenococcum geophilum	N/A	N/A	N/A	N/A	Both	7.7	11	15	12	7.3
Cortinarius 1	*Cortinarius* cf. *sertipes*	AJ889969	680	96%	Both	0	0	0	0.3	0.1
Cortinarius 2	*Cortinarius traganus*	AF335546	811	96%	Both	0	0.3	1.5	0	0.6
Cortinarius 3	*Cortinarius traganus*	AF335546	775	96%	Both	0	1.2	0.1	0.2	0.1
Cortinarius 4	*Cortinarius traganus*	AF335546	742	95%	Both	0	0	1.6	0.6	0
Cortinarius 5	*Cortinarius humicola*	AY083191	642	91%	Both	0	0	0.5	0	0.1
Cortinarius 7	*Cortinarius parahumilis*	AF539731	586	92%	Fir	0	0	3.7	0	0
Cortinarius 9	*Cortinarius cephelixus*	AY174784	748	92%	Birch	0	0	0.9	0	0

Functional "Species" Name	Closest NCBI or UNITE BLAST Match	Accession Number	Total Base Pairs Aligned[1]	% Sim.[2]	Host	Mean Relative Abundance (%)				
						5 cc	26 b	26 cc	65 b	100 b
Cortinarius 12	*Cortinarius rapaceus*	AF289146	564	94%	Fir	0	0	1.4	0	0
Cortinarius 13	*Cortinarius traganus*	AF335546	775	95%	Fir	0	1.7	0	0	0
Cortinarius 16	*Cortinarius heterosporus*	AF268894	604	94%	Fir	0	0	0	1.0	0
Cortinarius armillatus	*Cortinarius armillatus*	AF037223	533	98%	Birch	0	0	0	3.3	0
Cortinarius balaustinus	*Cortinarius balaustinus*	AF389153	586	98%	Birch	0	0	0	0	1.5
Cortinarius flexipes	*Cortinarius flexipes*	AJ889971	666	98%	Birch	0	0	0	0	0.1
Cortinarius gentiles	*Cortinarius gentiles*	AF325589	488	98%	Fir	0	0	0	1.9	0
Cortinarius hemitrichus	*Cortinarius hemitrichus*	Durall[3]	~800	99%	Birch	0	0	0	1.4	0
Cortinarius melliolens	*Cortinarius melliolens*	AF389144	510	99%	Birch	0	1.5	0	0	0
Cortinarius porphyropus	*Cortinarius porphyropus*	AY714854	755	98%	Birch	0.3	0	0	0	0
Cortinarius cf. *sertipes*	*Cortinarius* cf. *sertipes*	AJ889969	738	99%	Both	0	0	0	0.1	0.2
Cortinarius umbilicatus	*Cortinarius umbilicatus*	U56032	464	100%	Both	0	0	0	1.0	0
Cortinarius spp. Total	N/A	N/A	N/A	N/A	Both	0.2	5.9	6.8	5.8	1.8
Hebeloma 2	*Hebeloma incarnatulum*	AF430291	876	96%	Birch	0	0.7	0	0	0
Hebeloma incarnatulum	*Hebeloma incarnatulum*	AF430291	828	99%	Both	0	5.5	0	0.8	0.6
Hebeloma velutipes	*Hebeloma velutipes*	AF430254	656	99%	Both	0	4.4	4.2	2.3	1.3
Hebeloma spp. Total	N/A	N/A	N/A	N/A	Both	0	10	4.2	3.2	1.9
Inocybe 1	*Inocybe godeyi*	AJ889954	368	95%	Both	0.4	0	2.3	0.8	2.8
Inocybe 2	*Inocybe* cf. *glabripes*	AJ889952	295	92%	Fir	0	0	0.4	0.9	0
Inocybe 3	*Inocybe abietis*	AY038311	199	98%	Both	0	0	0	2.9	0

Functional "Species" Name	Closest NCBI or UNITE BLAST Match	Accession Number	Total Base Pairs Aligned[1]	% Sim.[2]	Host	Mean Relative Abundance (%)				
						5 cc	26 b	26 cc	65 b	100 b
Inocybe 4	*Inocybe* cf. *glabripes*	AJ889952	164	100%	Birch	0	0	0.9	0	0
Inocybe 5	*Inocybe flocculosa*	AY228354	201	96%	Both	0	0	0.5	0	0
Inocybe 6	*Inocybe sierraensis*	AY239025	283	97%	Birch	0	0	0	0	0.2
Inocybe 8	cf. *Inocybe* sp.	AY751588	552	88%	Birch	0.4	0	0	0	0
Inocybe 9	*Inocybe pudica*	AY228341	475	97%	Fir	0	0	0	1.2	0
Inocybe 10	*Inocybe abietis*	AY038311	252	98%	Both	0.7	0	0	0	0
Inocybe 11	*Inocybe godeyi*	AF335452	218	97%	Fir	0	0	0	0.3	0
Inocybe 12	*Inocybe maculata*	DQ241778	165	100%	Birch	0	0	0	0	0.2
Inocybe 13	*Inocybe godeyi*	AJ889954	381	93%	Fir	0	0	0	0	2.4
Inocybe nitidiuscula	*Inocybe nitidiuscula*	AJ534934	735	100%	Birch	0	0	0	0.3	0
Inocybe spp. total	N/A	N/A	N/A	N/A	Both	1.2	0	3.4	5.1	4.1
Laccaria 1	*Laccaria amethystia*	AF539737	766	97%	Birch	3.4	0	0	0	0
Laccaria bicolor	*Laccaria bicolor*	AY254878	573	98%	Both	2.2	0	0	0	0
Laccaria spp. total	N/A	N/A	N/A	N/A	Both	4.1	0	0	0	0
Lactarius 1	*Lactarius uvidus*	AJ534936	775	96%	Birch	1.6	0	0	1.4	0.2
Lactarius 2	Morphotype only	N/A	N/A	N/A	Both	0	0	0	0	0.3
Lactarius 3	Morphotype only	N/A	N/A	N/A	Birch	2.6	0	0	0	0
Lactarius pallescens	*Lactarius pallescens*	Durall[3]	~650	99%	Birch	0	0	0.4	0	0
Lactarius pubescens	*Lactarius pubescens*	AY336958	688	99%	Birch	27	0	0.4	0	0
Lactarius rubrilacteus	*Lactarius rubrilacteus*	Durall[3]	~700	99%	Fir	0	2.1	2.2	8.9	0.8

Functional "Species" Name	Closest NCBI or UNITE BLAST Match	Accession Number	Total Base Pairs Aligned[1]	% Sim.[2]	Host	Mean Relative Abundance (%)				
						5 cc	26 b	26 cc	65 b	100 b
Lactarius scrobiculatus	*Lactarius scrobiculatus*	AF140263	690	98%	Both	0.5	8.1	2.2	4.8	2.8
Lactarius torminosus	*Lactarius torminosus*	AY336959	686	99%	Birch	0	5.6	12	9	11
Lactarius spp. total	N/A	N/A	N/A	N/A	Both	19	12	9.6	14	8.7
Leccinum scabrum	*Leccinum scabrum*	AF454583	628	97%	Birch	16	4.9	12	2.9	2.6
MRA	*Cadophora finlandia*	AY394885	614	99%	Both	1.9	4.5	1.9	1.2	0.1
Phallales 1	*Ramaria flavobrunnescens*	AY102864	165	100%	Both	0	2.3	2.8	0.1	0
Piloderma spp.	*Piloderma fallax*	AY010281	580	99%	Both	0	3.5	5.4	9.5	12
	Piloderma sp. B22	AJ534903	662	100%						
Rhizopogon rudus	*Rhizopogon rudus*	AF377107	611	98%	Fir	6.0	2.6	4.3	0	0.9
Rhizopogon vinicolor-type	*Rhizopogon vinicolor*	AF263933	697	99%	Fir	82	46	27	23	30
	Rhizopogon vesiculosus	AF262931	700	99%						
Rhizopogon spp. total	N/A	N/A	N/A	N/A	Fir	88	49	31	23	31
Russula 1	*Russula delica*	AY061671	613	95%	Both	0	0	0	4.8	0
Russula 2	*Russula gracillima*	AY061678	631	97%	Birch	0	6.5	0	0.5	0.9
Russula 3	*Russula gracillima*	AY061678	635	96%	Birch	0	0.9	1.8	0	0
Russula 5	*Russula* cf. *xerampelina*	AY228344	244	96%	Birch	0	0	1.4	0	0
Russula 6	*Russula ilicis*	AY061682	213	97%	Fir	0	0	2.6	0	0
Russula 7	*Russula ilicis*	AY061682	374	94%	Fir	0	0	0	0.6	0
Russula 8	*Russula adusta*	AY061652	645	95%	Birch	0	0	0	0.6	0
Russula aeruginea	*Russula aeruginea*	AF418612	323	99%	Both	0	0	1.4	1.5	4.5

Functional "Species" Name	Closest NCBI or UNITE BLAST Match	Accession Number	Total Base Pairs Aligned[1]	% Sim.[2]	Host	Mean Relative Abundance (%)				
						5 cc	26 b	26 cc	65 b	100 b
Russula brevipes	*Russula brevipes*	AF349714	607	99%	Both	0	0	0	0	6.0
Russula fragilis	*Russula fragilis*	Durall[3]	~700	100%	Fir	0	0	0	1.8	1.7
Russula nigricans	*Russula nigricans*	Durall[3]	~800	99%	Both	0	1.7	2.4	4.9	7.1
Russula postiana	*Russula postiana*	AF230898	600	98%	Both	0	1.0	0.6	4.6	0.2
Russula roseipes	*Russula roseipes*	AY061716	655	98%	Fir	0	0	0	0	3.6
Russula velenovskyi	*Russula velenovskyi*	AY061721	654	98%	Birch	0.9	0	0	4.1	0
Russula versicolor	*Russula versicolor*	AY061722	634	98%	Both	1.0	1.9	1.8	0.9	0
Russulaceae 1	*Gymnomyces monticola*	AY239313	645	93%	Both	0	0	0	2.3	6.5
Russula spp. total	N/A	N/A	N/A	N/A	Both	1.5	8.8	7.9	18	27
Sebacinaceae 1	*Sebacina* endomycorrhiza	AF440648	817	96%	Both	0	0	0	1.0	1.1
Sebacinaceae 2	*Sebacina* endomycorrhiza	AF440650	697	99%	Birch	0	0	1.2	0	0
Sebacinaceae 3	Uncultured ECM	AJ893264	471	97%	Birch	0	0	0	0	0.4
Sebacinaceae 4	*Sebacina* endomycorrhiza	AF440651	877	98%	Birch	0	0	0.3	0	0
Sebacinaceae spp. total	N/A	N/A	N/A	N/A	Both	0	0	0.7	1.0	1.3
Suillus lakei	*Suillus lakei*	L54086	627	98%	Fir	0	14	26	0.7	1.8
Thelephora terrestris	*Thelephora terrestris*	U83486	685	99%	Both	5.7	0	0	0	0
Thelephoraceae 1	*Pseudotomentella tristis*	UDB*279	419	93%	Birch	0	0	1.4	0	0
Thelephoraceae 3	*Thelephora terrestris*	UDB*215	483	91%	Fir	0	0	0	0.2	0
Thelephoraceae 4	*Tomentella laterita*	UDB*954	584	89%	Fir	0	0	0	0	0.7
Tomentella 1	*Tomentella coerula*	UDB*266	587	93%	Both	0	0.1	0	0.1	0.3

Functional "Species" Name	Closest NCBI or UNITE BLAST Match	Accession Number	Total Base Pairs Aligned[1]	% Sim.[2]	Host	Mean Relative Abundance (%)				
						5 cc	26 b	26 cc	65 b	100 b
Tomentella 2	*Tomentella bryophila*	UDB*035	607	95%	Fir	0	0	0	1.5	0.1
Tomentella 3	*Tomentella fuscocinerea*	UDB*240	584	94%	Both	0	0	0	0	0.4
Tomentella 4	*Tomentella viridula*	UDB*261	492	95%	Birch	0	0	0.5	0	0.4
Tomentella 5	*Tomentella atramentaria*	UDB*235	587	97%	Birch	0	1.1	0	0	0
Tomentella 6	*Tomentella atramentaria*	UDB*235	469	93%	Both	0	0	0	0.1	1.8
Tomentella 7	*Tomentella subclavigera*	UDB*259	468	96%	Both	0	0.7	2.2	1.2	0.4
Tomentella 8	*Tomentella subclavigera*	UDB*259	559	94%	Birch	2.1	0	0	0.6	0.4
Tomentella 9	*Tomentella lilacinogrisea*	UDB*272	556	94%	Birch	2.0	0	0	0	0
Tomentella 10	*Tomentella lilacinogrisea*	UDB*272	547	97%	Both	0.9	0.6	0.8	0	0.5
Tomentella 11	*Tomentella bryophila*	UDB*035	673	92%	Both	0	0	0	0	2.9
Tomentella 12	*Tomentella bryophila*	UDB*035	672	93%	Birch	0	0	0	1.1	0
Tomentella 13	*Tomentella subclavigera*	UDB*957	572	94%	Birch	0	0	0	0.1	0
Tomentella 14	*Tomentella badia*	UDB*961	578	97%	Fir	0	0	0	0.8	0
Tomentella 15	*Tomentella bryophila*	UDB*035	672	92%	Birch	0	1.6	0	0	0
Tomentella ramosissima	*Tomentella ramosissima*	U83480	615	98%	Both	0	0	0	0	0.3
	Tomentella lapida	UDB*250	582	99%						
Tomentella terrestris	*Tomentella terrestris*	AF272911	582	99%	Both	2.9	0	0	0.8	0.7
Thelephoraceae spp. total	N/A	N/A	N/A	N/A	Both	9.0	2.9	3.6	4.3	8.0
Tricholoma flavovirens	*Tricholoma flavovirens*	AF349689	462	98%	Birch	0	0	0	0.4	0
Tricholoma scalpaturatum	*Tricholoma scalpaturatum*	AF377199	413	99%	Birch	0	1.3	0	0	0

Functional "Species" Name	Closest NCBI or UNITE BLAST Match	Accession Number	Total Base Pairs Aligned[1]	% Sim.[2]	Host	Mean Relative Abundance (%)				
						5 cc	26 b	26 cc	65 b	100 b
Truncocolumella citrina	*Truncocolumella citrina*	L54097	653	99%	Fir	0	0	1.3	1.1	1.1
Tuber 1	*Tuber borchii*	AF106890	476	92%	Both	0.8	0.3	1.0	3.5	0.2
Wilcoxina rehmii	*Wilcoxina rehmii*	AF266708	536	99%	Both	0.7	0.1	0.2	0	0.2

[1] Includes only the longest aligned segment for alignments to a single taxon in which unaligned gaps were present.
[2] Percent similarity of sample sequence(s) to database sequence, ignoring gaps and unknown bases; respective of footnote [1].
[3] Sequences matched to sporocarps collected and identified in Durall *et al.* (2006)(see Chapter 2).

Appendix C

This table lists correlations (Pearson's *r)* of stand age and species to NMS ordination axes. "Frequency" and "Abundance" denote the type of data input used to generate ordinations. "By Species" ordinations included species groups in which not all occurrences were resolved to a unique genotype (i.e. there were two genotypes each in *Rhizopogon vinicolor*-type and *Piloderma* spp). "By Genera" ordinations included species that were the sole representatives of their genera in this study. Only species with a correlation with an absolute value of 0.5 or higher to at least one ordination axis are listed (correlations of 0.5 or larger absolute value are in bold).

	Both hosts; Frequency; By Species		**Both hosts; Frequency; By Genera**		**Both hosts; Abundance; By Species**		**Douglas-fir; Frequency; By Species**		**Douglas-fir; Frequency; By Genera**		**Douglas-fir; Abundance; By Species**		**Paper birch; Frequency; By Species**		**Paper birch; Frequency; By Genera**		**Paper birch; Abundance; By Species**	
Axes	**1**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**1**	**3**	**1**	**2**	**2**	**3**
R^2	0.23	0.46	0.67	0.18	0.22	0.45	0.22	0.47	0.27	0.39	0.25	0.19	0.33	0.38	0.29	0.30	0.42	0.31
P (Monte Carlo)	0.02	0.02	0.02	0.02	0.04	0.02	0.06	0.02	0.02	0.02	0.24	0.53	0.02	0.04	0.02	0.02	0.04	0.04
Correlations with axes																		
Stand Age	-0.31	**0.88**	**0.84**	.02	-0.16	**-0.86**	**0.62**	0.29	**-0.75**	0.27	-0.46	0.25	**-0.69**	**-0.73**	**-0.67**	**0.74**	**-0.70**	0.25
Cenococcum geophilum	-0.39	-0.16	0.19	-0.14	0.40	-0.11	0.30	0.29	-0.07	0.09	0.20	-0.28	-0.21	-0.03	-0.36	-0.39	**-0.54**	-0.05
Cortinarius 2	-0.32	-0.13	N/A	N/A	0.25	0.05	-0.01	0.17	N/A	N/A	-0.21	-0.26	0.08	-0.20	N/A	N/A	-0.09	**0.51**
Cortinarius 4	-0.30	-0.05	N/A	N/A	0.44	0.03	-0.13	**0.53**	N/A	N/A	-0.46	-0.19	-0.10	-0.29	N/A	N/A	0.11	0.30
Cortinarius 5	0.07	0.30	N/A	N/A	-0.04	-0.04	0.06	-0.05	N/A	N/A	-0.33	**0.61**	-0.02	-0.02	N/A	N/A	-0.27	-0.03
Cortinarius cf. *sertipes*	-0.02	**0.62**	N/A	N/A	-0.34	-0.47	0.06	-0.05	N/A	N/A	-0.33	**0.61**	-0.36	-0.12	N/A	N/A	-0.29	0.03
Cortinarius spp.	N/A	N/A	0.46	-0.24	N/A	N/A	N/A	N/A	**-0.78**	0.38	N/A	N/A	N/A	N/A	-0.41	-0.15	N/A	N/A
Hebeloma incarnatulum	0.23	0.16	N/A	N/A	0.06	0.01	0.06	-0.05	N/A	N/A	-0.33	**0.61**	-0.21	0.15	N/A	N/A	-0.21	-0.33
Hebeloma velutipes	**-0.69**	-0.09	N/A	N/A	0.07	-0.05	-0.04	0.45	N/A	N/A	-0.40	0.11	0.46	-0.39	N/A	N/A	0.36	0.36

	Both hosts; Frequency; By Species		Both hosts; Frequency; By Genera		Both hosts; Abundance; By Species		Douglas-fir; Frequency; By Species		Douglas-fir; Frequency; By Genera		Douglas-fir; Abundance; By Species		Paper birch; Frequency; By Species		Paper birch; Frequency; By Genera		Paper birch; Abundance; By Species	
Axes	**1**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**1**	**3**	**1**	**2**	**2**	**3**
R^2	0.23	0.46	0.67	0.18	0.22	0.45	0.22	0.47	0.27	0.39	0.25	0.19	0.33	0.38	0.29	0.30	0.42	0.31
P (Monte Carlo)	0.02	0.02	0.02	0.02	0.04	0.02	0.06	0.02	0.02	0.02	0.24	0.53	0.02	0.04	0.02	0.02	0.04	0.04
Correlations with axes																		
Stand Age	-0.31	**0.88**	**0.84**	.02	-0.16	**-0.86**	**0.62**	0.29	**-0.75**	0.27	-0.46	0.25	**-0.69**	**-0.73**	**-0.67**	**0.74**	**-0.70**	0.25
Inocybe 1	0.06	0.11	N/A	N/A	-0.05	-0.04	0.21	-0.23	N/A	N/A	0.01	0.16	-0.38	-0.09	N/A	N/A	**-0.52**	-0.17
Laccaria 1	0.49	-0.27	N/A	N/A	-0.29	0.40	N/A	N/A	N/A	N/A	N/A	N/A	-0.12	**0.50**	N/A	N/A	0.47	-0.45
Lactarius pubescens	**0.71**	-0.46	N/A	N/A	-0.40	.060	N/A	N/A	N/A	N/A	N/A	N/A	0.11	**0.74**	N/A	N/A	**0.51**	**-0.65**
Lactarius rubrilacteus	-0.33	0.30	N/A	N/A	0.16	-0.37	**0.61**	0.26	N/A	N/A	-0.03	-0.34	N/A	N/A	N/A	N/A	N/A	N/A
Lactarius scrobiculatus	-0.32	-0.28	N/A	N/A	**0.66**	-0.20	-0.27	**0.55**	N/A	N/A	-0.45	-0.33	0.17	-0.01	N/A	N/A	-0.18	**0.52**
Lactarius torminosus	**-0.54**	0.03	N/A	N/A	0.27	-0.45	N/A	N/A	N/A	N/A	N/A	N/A	0.25	-0.35	N/A	N/A	-0.01	**0.57**
Lactarius spp.	N/A	N/A	-0.33	0.29	N/A	N/A	N/A	N/A	0.01	**0.51**	N/A	N/A	N/A	N/A	**0.57**	-0.36	N/A	N/A
Leccinum scabrum	-0.20	-0.40	0.31	-0.37	-0.23	0.46	N/A	N/A	N/A	N/A	N/A	N/A	**0.69**	0.20	**0.60**	-0.14	**0.70**	-0.34
MRA	-0.23	**-0.60**	0.46	**-0.50**	0.35	0.28	-0.21	0.24	0.08	0.23	-0.11	-0.39	**0.67**	0.20	**0.58**	-0.47	0.14	-0.38
Phallales 1	**-0.53**	-0.31	-0.11	**-0.69**	**0.57**	0.14	-0.15	**0.64**	0.15	**0.72**	-0.41	-0.39	0.28	-0.21	0.14	-0.12	0.11	0.19
Piloderma spp.	**-0.57**	**0.82**	**0.81**	0.06	-0.18	**-0.78**	**0.83**	0.47	-0.43	0.38	-0.27	-0.48	**-0.63**	**-0.68**	**-0.62**	**0.77**	**-0.57**	-0.23
Rhizopogon vinicolor-type	**0.69**	-0.44	N/A	N/A	-0.40	**0.79**	**-0.50**	**-0.64**	N/A	N/A	**0.75**	0.19	N/A	N/A	N/A	N/A	N/A	N/A
Rhizopogon spp.	N/A	N/A	**-0.52**	0.47	N/A	N/A	N/A	N/A	**0.55**	**-0.65**	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Russula brevipes	0.01	**0.55**	N/A	N/A	-0.44	-0.36	0.34	0.02	N/A	N/A	-0.33	**0.60**	**-0.59**	-0.19	N/A	N/A	**-0.50**	-0.22
Russula fragilis	0.05	**0.60**	N/A	N/A	-0.47	-0.28	0.28	-0.10	N/A	N/A	-0.32	**0.53**	N/A	N/A	N/A	N/A	N/A	N/A
Russula nigricans	-0.14	0.39	N/A	N/A	0.13	-0.26	-0.32	0.01	N/A	N/A	-0.18	0.01	-0.47	**-0.51**	N/A	N/A	**-0.57**	0.12
Russula roseipes	0.06	0.45	N/A	N/A	-0.42	-0.39	0.06	-0.05	N/A	N/A	-0.33	**0.61**	N/A	N/A	N/A	N/A	N/A	N/A
Russula versiclolor	-0.25	-0.25	N/A	N/A	0.34	0.16	-0.10	**0.52**	N/A	N/A	-0.44	-0.25	0.31	-0.06	N/A	N/A	0.19	0.22

	Both hosts; Frequency; By Species		**Both hosts; Frequency; By Genera**		**Both hosts; Abundance; By Species**		**Douglas-fir; Frequency; By Species**		**Douglas-fir; Frequency; By Genera**		**Douglas-fir; Abundance; By Species**		**Paper birch; Frequency; By Species**		**Paper birch; Frequency; By Genera**		**Paper birch; Abundance; By Species**	
Axes	**1**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**1**	**3**	**1**	**2**	**2**	**3**
R^2	0.23	0.46	0.67	0.18	0.22	0.45	0.22	0.47	0.27	0.39	0.25	0.19	0.33	0.38	0.29	0.30	0.42	0.31
P (Monte Carlo)	0.02	0.02	0.02	0.02	0.04	0.02	0.06	0.02	0.02	0.02	0.24	0.53	0.02	0.04	0.02	0.02	0.04	0.04
Correlations with axes																		
Stand Age	-0.31	**0.88**	**0.84**	.02	-0.16	**-0.86**	**0.62**	0.29	**-0.75**	0.27	-0.46	0.25	**-0.69**	**-0.73**	**-0.67**	**0.74**	**-0.70**	0.25
Russulaceae 1	-0.21	**0.58**	N/A	N/A	**-0.57**	-0.45	0.26	0.02	N/A	N/A	-0.42	**0.60**	-0.11	**-0.51**	N/A	N/A	-0.08	0.26
Russula spp.	N/A	N/A	**0.85**	-0.30	N/A	N/A	N/A	N/A	**-0.90**	0.27	N/A	N/A	N/A	N/A	-0.33	**0.50**	N/A	N/A
Sebacinaceae 1	0.01	**0.61**	N/A	N/A	-0.10	**-0.55**	0.42	0.04	N/A	N/A	0.16	0.37	**-0.71**	-0.23	N/A	N/A	-0.38	-0.26
Suillus lakei	-0.44	-0.43	-0.17	**-0.59**	**0.65**	0.18	-0.43	**0.61**	0.12	**0.68**	**-0.50**	-0.42	N/A	N/A	N/A	N/A	N/A	N/A
Thelephora terrestris	**0.54**	-0.43	**-0.53**	0.36	-0.23	**0.55**	N/A	N/A	N/A	N/A	N/A	N/A	0.40	**0.54**	**0.57**	-0.22	0.46	-0.36
Tomentella 1	-0.32	0.24	N/A	N/A	-0.17	-0.22	0.41	0.08	N/A	N/A	0.02	-0.19	0.11	-0.47	N/A	N/A	0.14	0.39
Tomentella 2	-0.25	0.32	N/A	N/A	0.14	-0.33	**0.57**	0.44	N/A	N/A	-0.08	-0.28	N/A	N/A	N/A	N/A	N/A	N/A
Tomentella 3	0.37	-0.01	N/A	N/A	-0.20	-0.12	-0.21	-0.25	N/A	N/A	0.30	0.08	**-0.50**	-0.12	N/A	N/A	-0.45	-0.15
Thelephoraceae spp.	N/A	N/A	0.49	0.16	N/A	N/A	N/A	N/A	-0.44	-0.03	N/A	N/A	N/A	N/A	-0.23	**0.62**	N/A	N/A
Truncocolumella citrina	-0.32	**0.54**	**0.62**	-0.30	-0.17	-0.17	0.34	0.16	**-0.76**	-0.26	**-0.55**	0.03	N/A	N/A	N/A	N/A	N/A	N/A
Tuber 1	0.32	-0.27	0.03	-0.20	0.31	-0.24	**0.51**	0.27	0.02	0.27	0.10	0.32	0.17	0.12	0.32	-0.27	0.22	-0.33

Appendix D

This table gives a species list and web-based alignment/match information for ECM community of Douglas-fir seedlings observed in 5-yr-old stands. Base pairs aligned and percent similarity as per Appendix B.

Functional Species Name	Closest NCBI or UNITE BLAST Match	Accession Number	Base Pairs Aligned	% Sim.	Mean Relative Abundance (%)	
					Burned	Clearcut
Amphinema byssoides	See Appendix A	----------	----------	------	3.9	2.2
Boletus calopus	*Boletus calopus*	AJ889928	654	99%	2.3	0
Cenococcum geophilum	N/A	N/A	N/A	N/A	0.75	0
Cortinarius 6	*Cortinarius fusisporus*	AY254877	266	97%	0	1.6
Hebeloma 1	*Hebeloma testaceum*	AY320395	272	99%	0	1.2
Inocybe 4	See Appendix A	----------	----------	------	1.7	0
Lactarius rubrilacteus	See Appendix A	----------	----------	------	0	3.1
MRA	See Appendix A	----------	----------	------	0	0.16
Phallales 1	See Appendix A	----------	----------	------	0	0.71
Piloderma fallax	See Appendix A	----------	----------	------	0	0.71
Rhizopogon rudus	See Appendix A	----------	----------	------	37	10
Rhizopogon vinicolor-type	See Appendix A	----------	----------	------	35	67
Russula nigricans	See Appendix A	----------	----------	------	0	5.6
Russulaceae 1	See Appendix A	----------	----------	------	0	2.1
Suillus lakei	See Appendix A	----------	----------	------	1.1	0.2
Thelephora terrestris	See Appendix A	----------	----------	------	3.6	3.0
Thelephoraceae 1	See Appendix A	----------	----------	------	2.0	0
Tomentella 5	See Appendix A	----------	----------	------	2.3	0
Tomentella 10	See Appendix A	----------	----------	------	0.20	0
Truncocolumella citrina	See Appendix A	----------	----------	------	1.6	1.1
Tuber 1	See Appendix A	----------	----------	------	4.9	2.0
Wilcoxina rehmii	See Appendix A	----------	----------	------	0	1.4

Appendix E

This table gives a species list and web-based alignment/match information for ECM community of paper birch observed in soil associated with seedlings (clearcut and burned) and soil samples from spring (burned only) 5-yr-old stands. Percent similarity as per Appendix B.

Functional Species Name	Closest NCBI or UNITE BLAST Match	Accession Number	Base Pairs Aligned	% Sim.	Present (P) or Absent (A)	
					Burned	Clearcut
Boletus 1	See Appendix A	----------	----------	------	A	P
Cenococcum geophilum	N/A	----------	----------	------	P	P
Cortinarius spilomius	*Cortinarius spilomius*	Durall *et al.* 2006	~650	99%	A	P
Inocybe 7	*Inocybe dulcimara*	UDB0001196	627	96%	P	A
Lactarius pubescens	See Appendix A	----------	----------	------	P	P
Lactarius torminosus	See Appendix A	----------	----------	------	A	P
Leccinum scabrum	See Appendix A	----------	----------	------	P	P
MRA	See Appendix A	----------	----------	------	P	P
Phallales 1	See Appendix A	----------	----------	------	A	P
Russula velenovskyi	See Appendix A	----------	----------	------	P	A
Tomentella 10	See Appendix A	----------	----------	------	A	P
Tomentella terrestris	See Appendix A	----------	----------	------	P	P
Wilcoxina rehmii	See Appendix A	----------	----------	------	P	P