The effect of decayed or downed wood on the structure and function of ectomycorrhizal fungal communities at a high elevation forest

by

Jennifer Karen Marie Walker

B.Sc., The University of Northern British Columbia, 2003
M.Sc., The University of Northern British Columbia, 2006

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

The College of Graduate Studies

(Biology)

THE UNIVERSITY OF BRITISH COLUMBIA
(Okanagan)

March 2012

Abstract

Shifts in ectomycorrhizal (ECM) fungal community composition occur after clearcut logging, resulting in the loss of forest-associated fungi and potential ecosystem function. Coarse woody debris (CWD) includes downed wood generated during logging; decayed downed wood is a remnant of the original forest, and important habitat for ECM fungi. Over the medium term, while logs remain hard, it is not known if they influence ECM fungal habitat. I tested for effects of downed wood on ECM fungal communities by examining ECM roots and fungal hyphae of 10-yr-old saplings in CWD retention and removal plots in a subalpine ecosystem. I then tested whether downed and decayed wood provided ECM fungal habitat by planting nonmycorrhizal spruce seedlings in decayed wood, downed wood, and mineral soil microsites in the clearcuts and adjacent forest plots, and harvested them 1 and 2 years later. I tested for differences in the community structure of ECM root tips (Sanger sequencing) among all plots and microsites, and of ECM fungal hyphae (pyrosequencing) in forest microsites. I assayed the activities of eight extracellular enzymes in order to compare community function related to nutrient acquisition.

The retention of CWD caused a shift in ECM root tip fungal species composition on saplings at the plot scale within 12 years of clearcutting. Decayed wood and hard downed wood also provided habitat for some ECM fungal species. Abiotic

conditions in decayed wood and near downed wood on clearcuts were most similar to forest soils, but I did not detect a shift in ECM root tip or ECM hyphae community composition or function among microsites. Instead, ECM fungus community structure and enzyme activity differed most between clearcut and forest plots, and among forest plots. I could not determine if ecosystem function, in terms of soil macromolecule breakdown by ECM fungi, was maintained in clearcuts. *Amphinema byssoides*, *Thelephora terrestris*, and *Tylospora asterophora* were consistently the most abundant ECM taxa at Sicamous Creek. With pyrosequencing of fungal DNA, I was able to identify more ECM fungal taxa than in my previous experiments at this site. I concluded that CWD on clearcut blocks provides habitat for ECM fungi.

Preface

A version of Chapter 2 has been accepted by Applied Soil Ecology (February 22, 2012): Walker, J. K. M., Ward, V., Paterson, C., Jones, M.D. Coarse woody debris retention in subalpine clearcuts affects ectomycorrhizal root tip community structure within fifteen years of harvest. The original experimental design was part of a Forest Science Program grant written by M.D. Jones. I was responsible for harvesting the mesh bags, additional molecular identification of fungi on root tips and all molecular work on mesh bags, culturing and cloning of *Alloclavaria purpurea*, data analysis of all results but those related to *A. purpurea*, and writing the manuscript. Collection of the sapling roots, construction and burial of the mesh bags, morphotyping and molecular identification of some of the root tips, was performed by Valerie Ward. Courtney Paterson and Melanie Jones were responsible for all but the culturing and cloning portion of the of *A. purpurea* experiment. Melanie Jones contributed substantially to editing of the manuscript. I have retained the 'we', 'us', and 'our' throughout Chapter 2 to reflect the language used in the manuscript for work done in this collaboration. In all references to the work done in subsequent chapters, I have used 'I', 'me', and 'my'.

Field work for Chapter 3 required the help of many people. Specifically, Valerie Ward, Fawn Ross, Maryann Olson, and Brendan Twieg assisted in the planting

of hundreds of seedlings; Corey Anderson, Kate Sidlar, Ayla Fortin, and Natasha Lukey assisted in their harvesting. Lab work for Chapter 3 also required additional personnel. Specifically, Valerie Ward and Natasha Lukey assisted in performing the enzyme assays. I designed and implemented this experiment, and parts of it were used for Chapter 4. I was responsible for growing, planting, and harvesting the seedlings, installing, maintaining, and downloading the dataloggers, morphotyping and molecularly identifying the root tips for community analysis and enzyme assays, performing the enzyme assays, all data analysis, and writing the chapter. Jason Pither contributed crucial comments on the data analysis, while Melanie Jones contributed to editing of the chapter.

I was accompanied during seedling and substrate collection for Chapter 4 by Cynthia Wonham, Bailey Nicholson, Jeremy Bougoure, and Lori Ann Phillips. Lori Ann Phillips optimized lab protocols for soil assays and pyrosequencing, and analysed the carbon fraction of substrate samples. I was responsible for seedling and substrate collection, drying, grinding and preparing substrate samples for chemical analysis, performing pH tests, all DNA extraction and sample preparation for pyrosequencing, substrate enzyme assays, all data analysis, and writing the chapter. Melanie Jones contributed to editing of the chapter, and valuable input was added to this and all chapters by committee members Jason Pither, Louise Nelson, Craig Nichol, and Dan Durall.

Table of Contents

List of Tables

List of Figures

Acknowledgements

I thank all the members of my thesis supervisory committee for their expertise and insight, and especially Dr. Melanie Jones for her unflagging support.

I also thank the post-docs, students and technicians that helped me with so many aspects of field and lab work, especially Valerie Ward for her limitless patience.

For financial support I thank The University of British Columbia for Scholarships, Fellowships, Awards and Travel Grants.

For unconditional moral support I thank my outstanding friends and family.

1 Introduction

1.1 Experimental context based on the current literature

Branches and trees frequently fall to forest floors as a result of wind, age, disease, fire, and logging (Bunnell and Houde, 2010). Intact logs and branches provide shelter for and aid the travel of small mammals (Bunnell and Houde, 2010; Craig et al., 2006), and their surfaces are important substrates for non-vascular plants, lichens (Arsenault, 2002; Jonsson et al., 2005), and some resupinate fungi (Olsson et al., 2011; Tedersoo et al., 2003). Downed logs also influence the environment for soil organisms, including ectomycorrhizal (ECM) fungi (Elliot et al., 2007; Harvey et al., 1979; Tedersoo et al., 2003). While the wood is still hard (i.e. up to 15 years), large pieces of woody debris change the abiotic properties of the soil by moderating soil temperature fluctuations (Bunnell and Houde, 2010). Over hundreds of years, decaying logs gain and retain moisture (Bütler et al., 2007), accumulate N and P (Bütler et al., 2007; Laiho and Prescott, 1999), contribute to a thicker forest floor and upper soil horizon (Kayahara et al., 1996; Laiho and Prescott, 2004), and increase the amount of organic C in the soil in the immediate vicinity (Kayahara et al., 1996; Laiho and Prescott, 1999; Spears et al., 2003; Spears and Lajtha, 2004). Decaying logs are so important in forest ecosystems, that several jurisdictions have legislation requiring retention of coarse woody debris (CWD), usually defined as downed wood larger than 8-10 cm in diameter (Stevens, 1997).

Sufficiently decayed downed wood is penetrated by the roots of tree seedlings, and their ECM fungal symbionts (Christy et al., 1982; Harmon et al., 1986). Ectomycorrhizae are an integral part of most temperate forest soil ecosystems, and a diverse suite of ECM fungi is one feature of a healthy forest (Amaranthus et al., 1994). One of the most important influences of ectomycorrhizae on their hosts is to increase the uptake of poorly soluble mineral and organic nutrients by host trees (Read and Perez-Moreno, 2003; Smith and Read, 2008). They do this by secreting enzymes that break down recalcitrant organic molecules, and by absorbing nutrients in soil beyond the rhizosphere. Fungal mycelia that extend from the ECM mantle into the soil (extramatrical hyphae) are the principal structures involved in extracellular enzyme activity, nutrient and water acquisition and transport (Anderson and Cairney, 2007; Genney et al., 2006). Colonized root tips are the sites of fungus-plant contact and bidirectional transfer of nutrients and carbon (Smith and Read, 2008). It is essential that studies of community structure consider both parts of the system (hyphae inside root tips and extramatrical hyphae) because the distribution of ECM root tips colonized by a fungus and the extramatrical hyphae of that fungus do not necessarily coincide (Genney et al., 2006).

Shifts in ECM fungal community composition occur after clearcut logging, resulting in the loss of some ECM fungi that dominate undisturbed forest communities (Dickie et al., 2009; Jones et al., 2003; Mah et al., 2001). Decaying CWD is a remnant of the original forest (Elliott et al*.,* 2007), and may act to retain

and recruit old-growth-associated ECM fungi (and other soil microbes) if it is left behind after clearcut logging. For example, some ECM genera decline markedly between mature forests and young disturbed stands where CWD is limited or absent (Smith et al., 2000), despite having abundant mycelia in mineral soils (Landeweert et al., 2003). *Piloderma* spp. (Goodman and Trofymow, 1998), other resupinate members of the ECM orders Thelephorales and Atheliales (Elliott et al., 2007; Tedersoo et al., 2003), and especially Tomentelloid species (Iwánski and Rudawska, 2007; Tedersoo et al., 2003, 2010b) are dominant on ECM root tips in, and on surfaces of, decayed wood. This habitat is not exclusive to cryptic ECM fungal species, however, as ectomycorrhizae of stipitate ECM fungi (e.g. *Laccaria, Tricholoma*) are also found in advanced stages of decaying wood (Iwánski and Rudawska, 2007; Tedersoo et al., 2010b). It is possible that CWD generated during, and subsequently retained after logging can provide a legacy of diverse ECM fungal inoculum to the regenerating stand, since the pool of ECM fungi that colonizes seedlings in forests is different from that in clearcuts (Dickie et al., 2009; Ding et al., 2011; Jones et al., 2003; Mah et al., 2001). The ECM fungal community that develops in a young stand regenerating after clearcut harvesting will be limited by the type and kind of inoculum available. For example, mycorrhizae of mature trees and spores dispersing in from the adjacent forest will be sources of inoculum only at the edges of clearcuts (Dickie and Reich, 2005; Hagerman et al., 1999; Peay et al., 2010; Tedersoo et al., 2008). Resistant propagules, such as spores and sclerotia, will not include the same

range of fungal species present in the forest (Izzo et al., 2006; Taylor and Bruns, 1999).

In intact systems, the extensive hyphal networks of soil fungi are important for releasing degradative enzymes and subsequently absorbing nutrients (Deacon, 2006), but the secretion of hydrolytic and oxidative enzymes by ECM fungi also contribute substantially to nutrient cycling (Luis et al., 2005; Molina et al., 2008; Schimel and Bennett, 2004). Fungal saprotrophs break down complex forms of dead organic matter, including wood, and most mycorrhizal fungi can provide their host plant with mineral nutrients such as nitrogen (N) and phosphorus (P) (Deacon, 2006). Soil fungi can break down simple polysaccharides and complex carbohydrates due to constitutively expressed (Deacon, 2006) and inducible (Courty et al., 2007) extracellular enzymes such as hemicellulases and cellulases (Cooke and Whipps, 1993). Laccases are thought to play important initial roles as redox molecules in lignin degradation by fungi (Webster and Weber, 2007), and are both constitutively expressed and inducible by temperature, pH changes, and the presence of plant cell wall compounds (Courty et al., 2007). The amino-sugar chain of chitin is broken down by chitinases in many fungal groups, including ECM fungi (Buée et al., 2007; Cooke and Whipps, 1993; Courty et al., 2007), providing both a source of carbon and of nitrogen (Carlile et al., 2001). Ectomycorrhizal fungi produce laccases (Baldrian, 2006), and are capable of degrading hemicellulose and cellulose (Smith and Read, 2008). They also produce aminopeptidases, and can assimilate organic (Aerts, 2002; Caldwell,

2005; Carlile et al., 2001), and inorganic (Smith and Read, 2008; Deacon, 2006) forms of N. Ectomycorrhizal fungi also produce phosphatases to cleave inorganic P from organic molecules (Caldwell, 2005; Leake et al., 2002; Smith and Read, 2008).

ECM fungi differ in their ability to mobilize nutrients (Jones et al., 2009; Smith and Read, 2008). The study of enzyme profiles associated with ECM fungi can provide information on the functional diversity of an ECM fungal community (Rineau and Courty, 2011). A system is said to be highly resilient, in the sense that it is capable of responding to a disturbance, if it can regain (due to trait plasticity) or maintain (due to functional overlap) its original stable state despite the loss of some species (Botton et al., 2006). Functional similarity (or redundancy) may be achieved if the system is species rich, or due to the presence of a few key species with large fundamental niches (Botton et al., 2006). If each taxon in a diverse system is restricted to a unique realized niche, then the system is considered highly specialized, and exhibits functional complementarity (Botton et al., 2006). Ectomycorrhizal fungal communities in forests are species rich, functionally similar, and demonstrate physiological plasticity for exoenzymes involved in organic matter breakdown (Buée et al., 2007; Rineau and Courty, 2011). Ectomycorrhizal fungal root tip communities have also demonstrated complementarity among taxa for organic matter depolymerases (Buée et al., 2007; Courty et al., 2007; Jones et al., 2009, 2010, in review). Nevertheless, the functional diversity of ECM fungal communities and

especially of individual ECM fungal taxa among soil microsites is not known. This knowledge may be valuable for management of disturbed systems, if it means that the loss of taxa that possess distinct enzyme profiles in their original forest habitat, results in decreased fitness for seedlings regenerating in a clearcut. Conversely, it would be equally important to know if dominant early successional species occupying a species-poor environment such as a clearcut possess a range of depolymerase activities. This could potentially preserve the overall physiological function of the ECM community regardless of the loss of some forest taxa even though functional similarity and resilience is usually associated with high-diversity communities (Peay et al., 2008). Locally diverse communities are in turn associated with heterogeneous habitats (i.e. that provide many different niches) (Bruns, 1995), and this is relevant for the post-harvest conditions in a clearcut.

Functional traits involved in resource uptake (such as the ability to produce fungal exoenzymes) are considered plastic, meaning that they can change based on the underlying habitat conditions, reflecting the realized niche of a species (Berg and Ellers, 2010). These types of physiological traits, and the capacity for a species to express them, are the backbone of niche-based community assembly (Berg and Ellers, 2010; Koide et al., 2011; Messier et al., 2010). While chance may underpin many apparent community patterns (McGill et al., 2006), and priority effects (Dickie et al., 2012; Fukami et al., 2010; Kennedy et al., 2009), the identity of available hosts (Ding et al., 2011; Tedersoo et al., 2008), and

competitive interactions (Kennedy et al., 2011; Koide et al., 2011) may at first determine fungal community assembly, potential enzyme activity can help to explain patterns in the mycorrhizal fungal community among distinct microsites (niches) (Peay et al., 2008; Parrent et al., 2010). This approach provides some insight into the realized physiological niche of these communities (and in some case of individual taxa), which are strongly and directionally shaped by many other interacting biotic and abiotic processes (Dickie, 2007; Dickie et al., 2009; Ding et al., 2011; Dumbrell et al., 2010; Koide et al., 2011; Parrent et al., 2010; Vellend, 2010). In addition, there are a number of stochastic (neutral) processes that contribute to the development of a mycorrhizal fungal community (Dumbrell et al., 2010), including dispersal limitation (Lekberg et al., 2007) and anthropogenic disturbance (Jones et al., 2003), by reducing species abundance in some locations (Vellend, 2010).

1.2 Site description, experimental design, and sampling scheme

Ectomycorrhizal fungal communities can be compared at scales ranging from centimeter-sized microsites to tens or even hundreds of meters at the stand level (Izzo et al., 2005, 2006; Lilleskov et al., 2004; Pickles et al., 2010). I investigated ECM fungal communities in decayed wood and mineral soil from clearcuts and undisturbed stands at the 150 ha Sicamous Creek Silvicultural Systems Trial, a high elevation forest located in the Engelmann spruce-subalpine fir wet-cold4 biogeoclimatic zone (ESSFwc4) which is characterized by long snowy winters. A

detailed description of this subzone can be found at http://www.for.gov.bc.ca/hre/becweb/resources/ classificationreports/ subzones/index.html. Unlogged areas are dominated by subalpine fir (*Abies lasiocarpa* Hook.) and Engelmann spruce (*Picea engelmannii* Parry ex. Engelm.) with a white rhododendron (*Rhododendron albiflorum*) and huckleberry/blueberry (*Vaccinium spp.*) understory (Craig et al., 2006). Soils are Humo-Ferric Podzols (Lloyd and Inselberg, 1997). Harvesting took place in the winter of 1994/95, with operational planting of Englemann spruce seedlings in the summer of 1996. Subalpine fir is regenerating naturally.

The experimental area includes three replicate 10 ha clearcut blocks (cutblocks) that range in elevation from 1583m to 1769m. These blocks are each within a larger 30 ha experimental unit, and are approximately 1 km apart. In each cutblock, two 1 ha treatment plots were established in the summer of 1995: one where coarse woody debris (large logs) generated during the harvesting was retained and one where as much coarse woody debris as possible was removed. This site work was part of a collaboration between the BC Ministry of Forests (Kamloops Region) and Riverside Forest Products Ltd. (Lumby Division) (Hollstedt and Vyse, 1997). Operational planting took place in these cutblocks the following summer (Figure 1.1). All of the experiments in Chapter 2 refer to the root systems of operationally planted spruce saplings in the retention and removal plots within the 10 ha clearcut blocks. In 2007, I established a 1 ha plot in the forest south of each cutblock, and planted 8-wk-old, non-mycorrhizal hybrid

P. engelmannii x *Picea glauca* (Moench) Voss (native interior hybrid spruce) seedlings in three types of microsites in all plots (Figure 1.2). I characterized the three microsites by the nature of their substrates: decayed wood, soil next to a hard intact log, and mineral soil as a control (Figure 1.3). When I harvested the seedlings one and two years later, I examined three substrate fractions: the mycorrhizoplane, mycorrhizosphere, and bulk substrate (Figure 1.4). The experiments in Chapter 3 refer to the fine roots of spruce seedlings planted in all three microsite types in retention, removal, and forest plots replicated over three blocks. The experiments in Chapter 4 refer to the substrate surrounding each of the spruce seedlings planted in all three microsite types in the three replicate forest plots only.

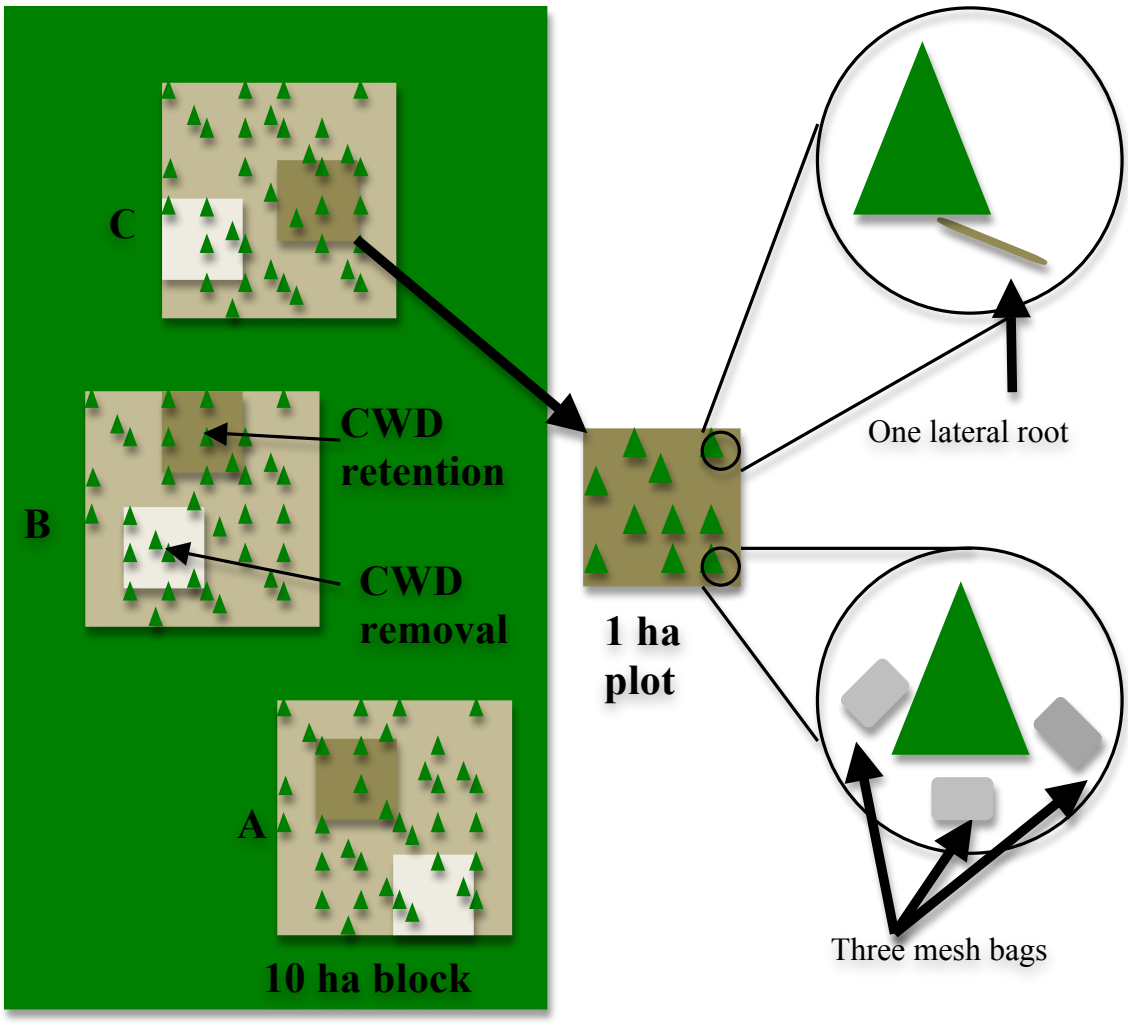


Figure 1.1 Plot-level experimental design (Chapter 2).
Three replicate 10-ha clearcut blocks (A, B, and C) each contain two 1-ha treatment plots (CWD retention and CWD removal). From each 1-ha plot, ten 10yr.-old operationally planted spruce saplings were randomly selected for each experiment. For ECM root tip community structure and enzyme activity, one lateral root was removed from each sampling; colonized root tip were then excised from the root for analysis. For ECM hyphae community structure, three sand-filled mesh bags were buried in the root system of each sapling; the bags were recovered after one year, and the sand was pooled per sapling. ECM root tips and mesh bags collected for community structure analysis were taken from the same saplings in different years (the former 2006, the latter 2007). All experiments in Chapter 2 refer to these samples and this experimental design.

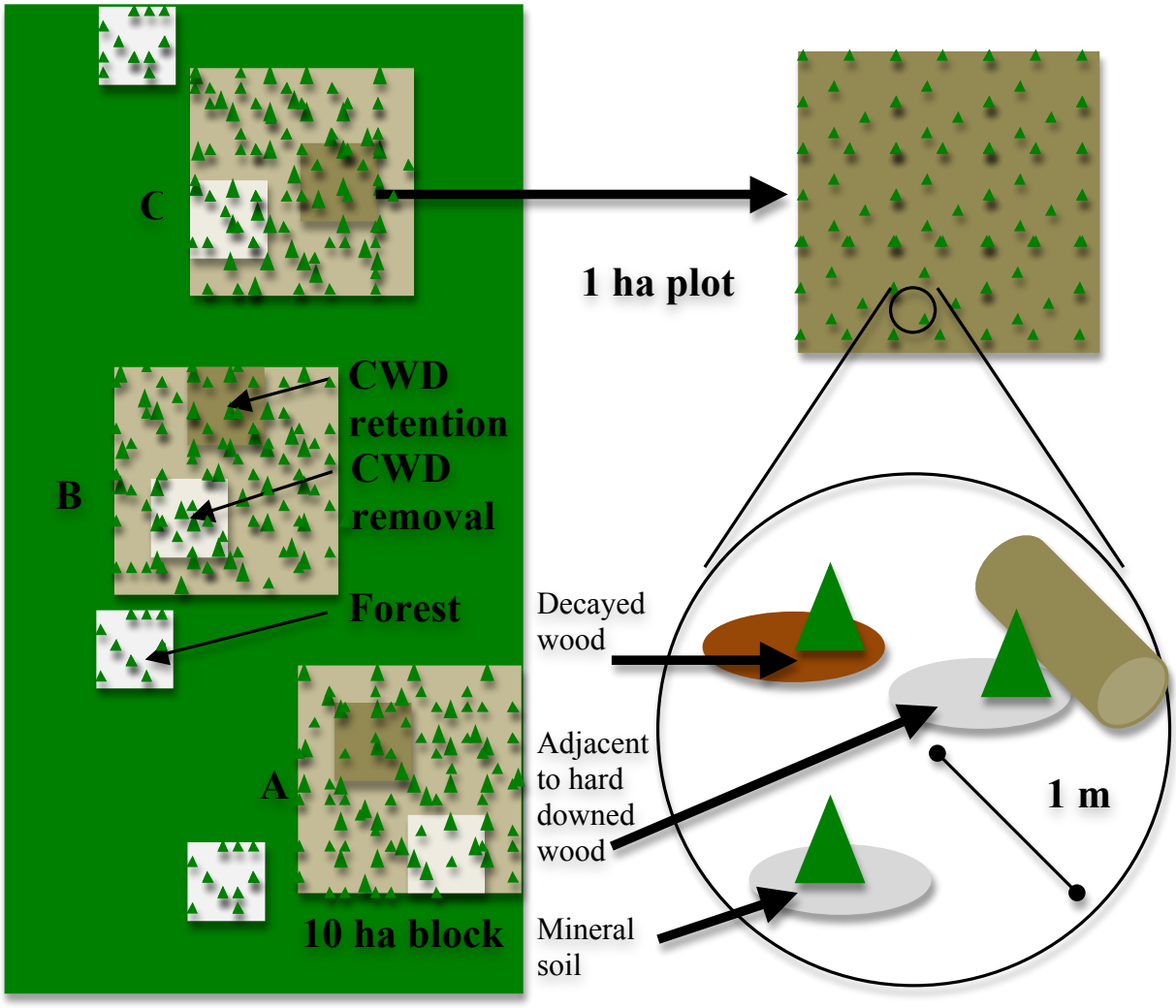


Figure 1.2 Microsite level experimental design (Chapters 3 and 4).
Three replicate 10-ha blocks (A, B, and C) each contain two 1-ha treatment plots (CWD retention and CWD removal) and have one adjacent 1-ha forest plot well inside the forest edge. Inside every plot, twenty-five random locations were chosen where 8wk.-old non-mycorrhizal spruce seedlings were planted in each of three microsites: decayed wood, soil next to a piece of hard downed wood, and mineral soil (as a control). The three substrates were visually identified based on predetermined criteria, and were within one meter of the randomly chosen spot. For each experiment, five seedlings – including entire root system and surrounding soil – were harvested from each microsite substrate in every plot. Two sets of five seedlings were selected one year after planting (2008): one set for ECM root tip community structure, and one set for enzyme activity. All experiments in Chapter 3 refer to these samples and this complete experimental design. One set of five 'seedlings-plus-soil' was collected the following year from the forest plots for fungal hyphae community structure and enzyme activity of soil. All experiments in Chapter 4 refer to these samples and this experimental layout in the forest plots only

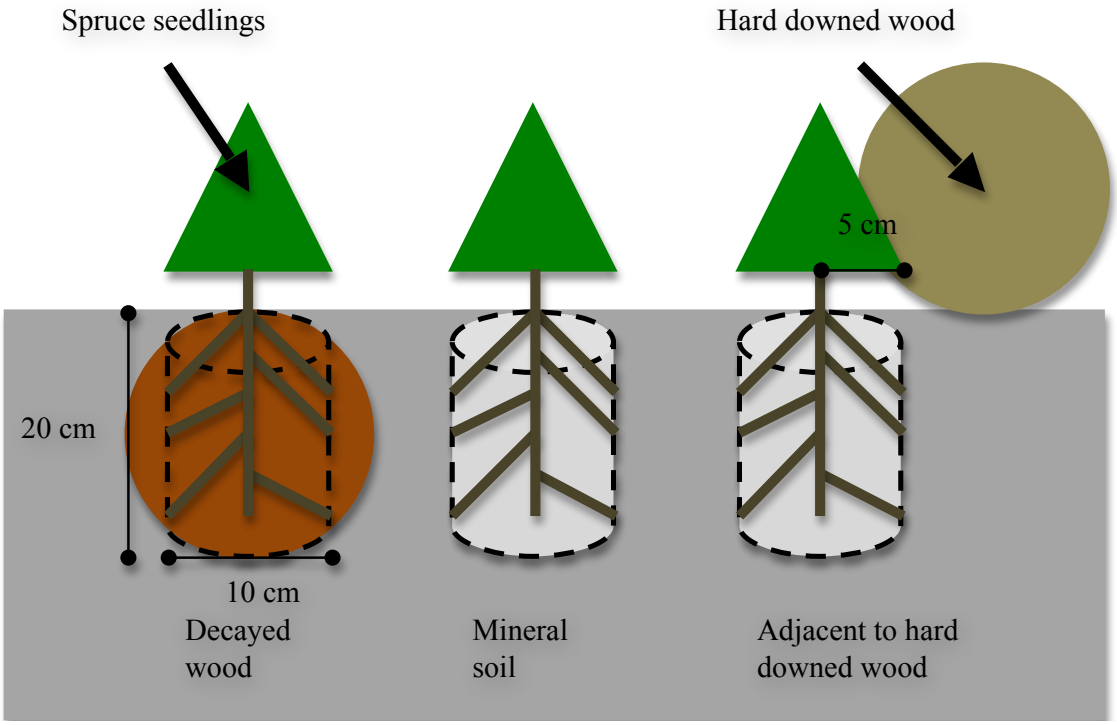


Figure 1.3 Microsite substrate and sampling details (Chapters 3 and 4).
Non-mycorrhizal spruce seedlings were planted at 8 weeks old, then harvested one- and two-years later. Decayed wood microsites were identified as having red, decayed (but not blocky) wood on the soil surface and when exposed by the planting tool. Mineral soil (used as a control) was at least 50 cm away from decayed wood and hard wood microsites. Hard downed wood was at least 10 cm in diameter, and with the entire log on the ground but no sagging. Most of the logs in the retention plots were without bark. The longest lateral roots of stumps were used instead of logs in removal plots. Soil cores collected were 20 cm deep and 10 cm in diameter in order to accommodated the entire seedling root system.

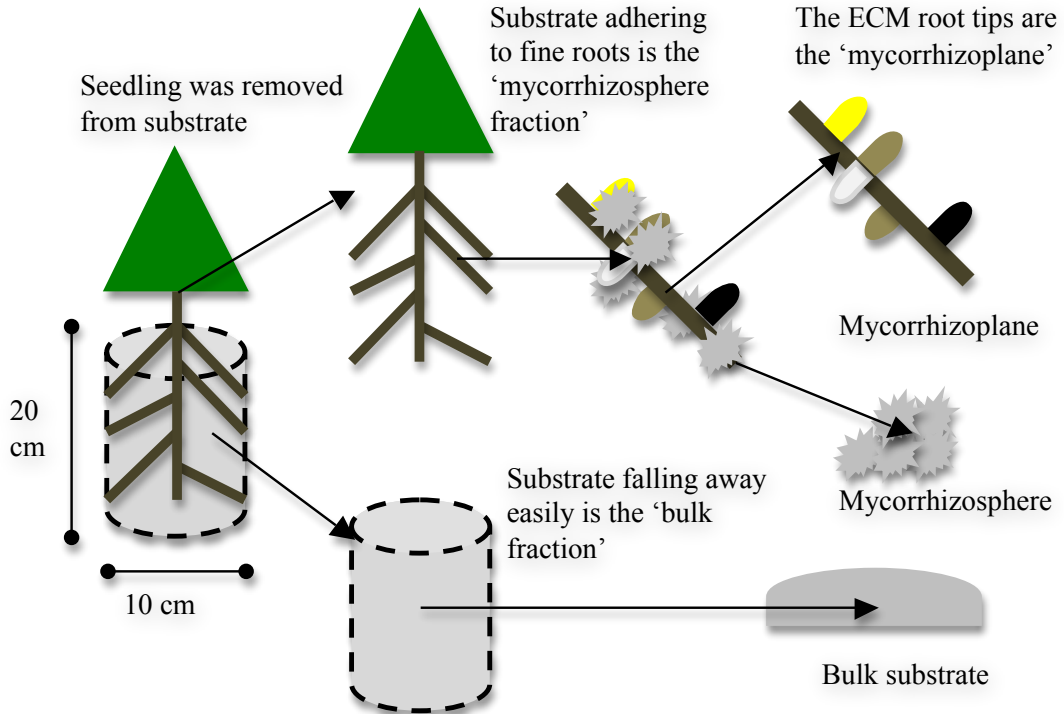


Figure 1.4 Substrate fraction details.
Soil/substrate cores containing entire seedling root systems were removed one and two years after planting. Seedling root systems were gently removed from their substrates, and all substrate that fell away easily was collected for the 'bulk fraction'. Substrate adhering to fine roots due to fungal hyphae was gently pulled away and collected for the 'mycorrhizosphere fraction'. The remaining fungal mantle of ECM root tips was designated the 'mycorrhizoplane' (Chapter 3). All three substrate fractions collected from forest plots in the second year will be analysed together in a future paper, but data from the bulk soil fraction are analysed in this thesis (Chapter 4).

1.3 Chapter objectives and hypotheses

1.3.1 Coarse woody debris retention in subalpine clearcuts affects the community structure of ectomycorrhizal fungi within fifteen years of harvest (Chapter 2).

Objective 1: To determine if there are differences in ECM fungal community structure (e.g. species richness or composition) between 1 ha CWD retention and CWD removal plots in clearcuts at Sicamous Creek.

Hypothesis 1: Taxonomic differences will not be found among ectomycorrhizae on sapling root systems between CWD treatments nor among ECM hyphae in sapling root zones between CWD treatments.

Prediction 1: If ECM fungal communities are affected by increased habitat diversity (i.e., created by shading or moisture retention, or by increased nutrient availability due to the hard downed wood) on 1 ha CWD retention plots, I expect greater species diversity (i.e. richness and evenness) and/or a shift in the community structure (e.g. a change in the frequency or relative abundance) of ectomycorrhizae on sapling root systems and of ECM hyphae in sapling root zones in CWD retention plots. *However, I predict that no taxonomic differences will be found between CWD retention and CWD removal treatments because the plot scale is larger than that of the variation among ECM communities, and because undecayed CWD does not modify the surrounding habitat.*

Objective 2: To compare ECM fungal communities to those found during initial studies of the ECM fungal community at Sicamous Creek to determine whether succession has occurred over the medium term (i.e. less than fifteen years after harvest).

Hypothesis 2: Succession in the ECM fungal community has occurred since the initial studies at this site.

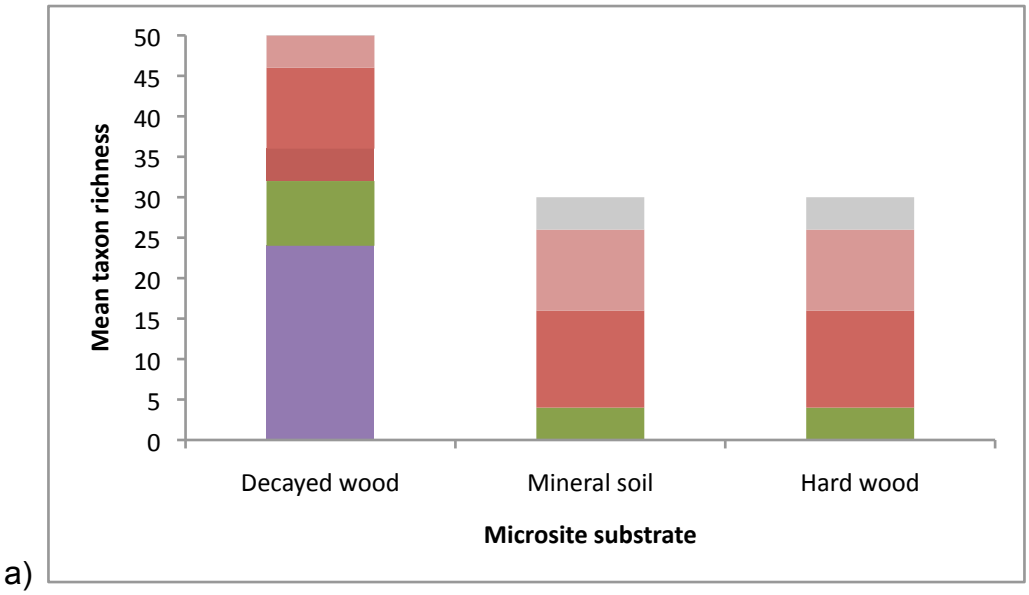
Prediction 2: I expect a change in the identity of the fungal symbiont of ectomycorrhizae on sapling root systems. *I predict that I will detect succession in the ECM fungal community after less than fifteen years post-harvest, because different ECM fungal species are known to occur soon after a disturbance, while others occur later on in succession.*

1.3.2 Ectomycorrhizal root tip community structure and enzyme activity varies among forest and clearcut plots, but not among decayed wood, downed wood, and mineral soil microsites (Chapter 3).

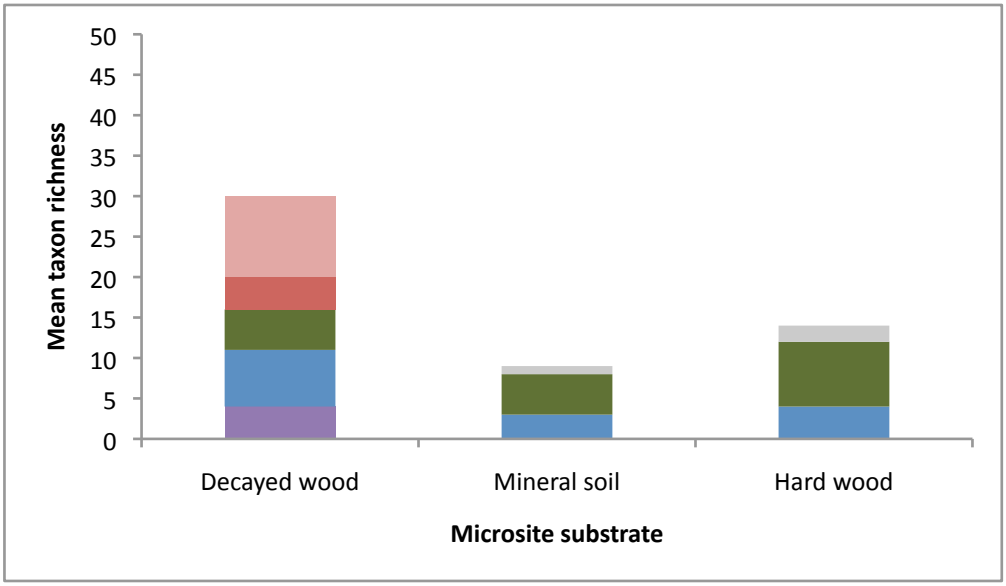
Objective 3: To determine whether the composition and physiological activities of ectomycorrhizae differ among microsites of decayed wood, mineral soil, or adjacent to hard downed wood in clearcuts, and their similarities to those in forest microsites.

Hypothesis 3: Taxonomic and functional differences will be found in the ECM root tip community among soil microsites, but those from decayed wood will be most similar to those in forest plots (Figures 1.5 and 1.6).

Prediction 3: If ectomycorrhizal root tip communities are structured by substrate properties (e.g. temperature or C availability), I would detect a shift in the fungal community among microsites of decayed wood, mineral soil, and hard downed wood. If the ability of the ECM fungi to access nutrients from organic molecules differed among substrates (i.e. a shift to those with cellulolytic abilities in the decayed wood), I would detect altered patterns of enzyme activity among the microsites and between the plots. *I predict that both taxonomic and functional differences will be found in the ECM root tip community because the substrates differ greatly in temperature, moisture and nutrient status, and because ECM communities are structured by these properties.*



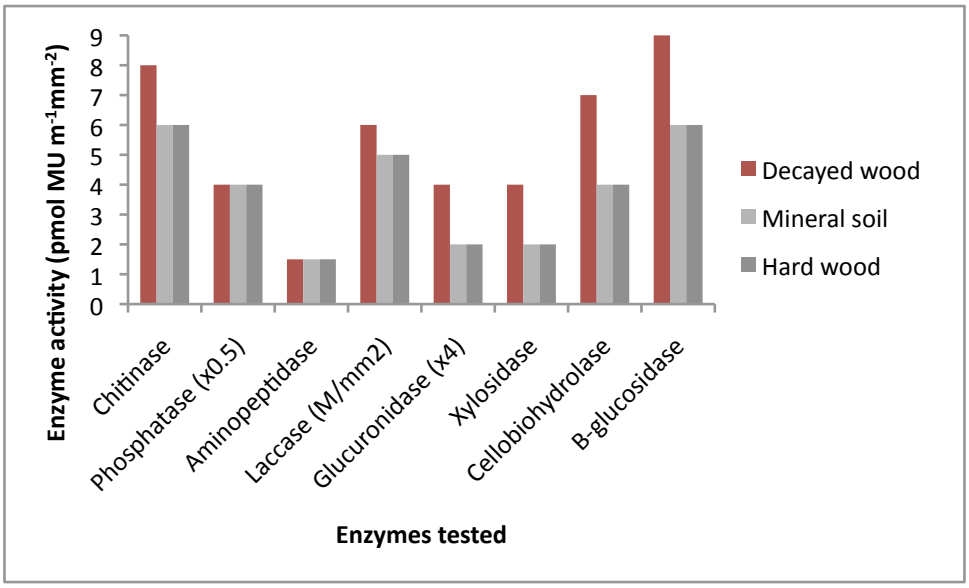
a)



b)

Figure 1.5 Mean taxon richness and relative abundance of major taxa in a hypothetical community at all three microsite substrates in a) forest plots, and b) clearcut plots as predicted by Hypothesis 3.
Few taxa will be shared between forest plots and clearcut plots, but the greatest sharing will be with decayed wood microsites in the clearcuts. Taxon richness will be higher overall in forest versus clearcut plots for all microsites; richness will also be higher in decayed wood microsites versus mineral soil and hard wood at both plots. Each colour represents one hypothetical taxon.

a)



b)

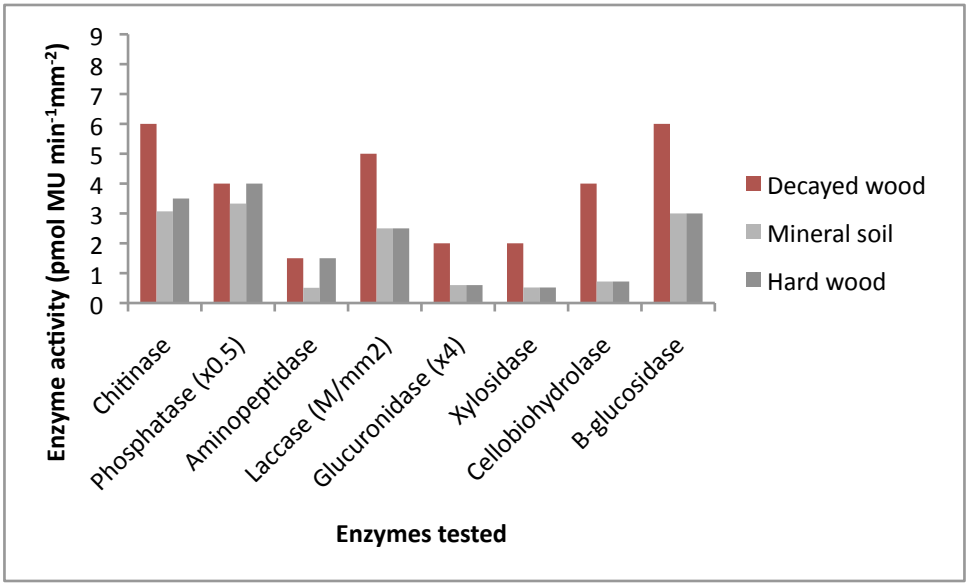


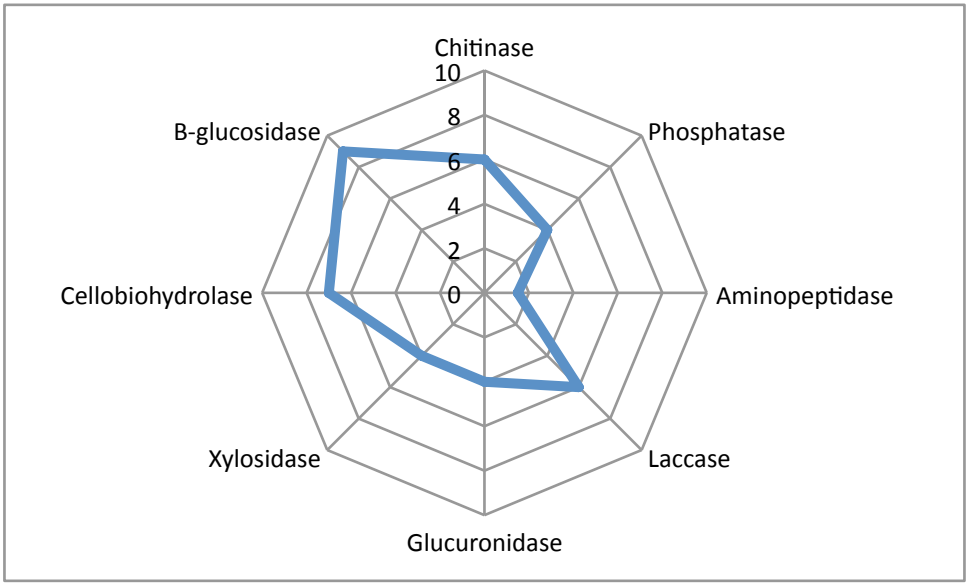
Figure 1.6 Hypothetical exoenzyme activity for eight enzymes tested in all three microsites at a) forest, and b) clearcut plots as predicted by Hypothesis 3.
Enzyme activity will be higher overall in microsites at forest plots; cellulolytic enzyme activity will be higher in decayed wood microsites than in mineral soil or hard wood at both plots. Enzyme activity will not differ between mineral soil and hard wood microsites in forest plots because conditions on the forest floor are similar. Levels of some enzymes will be higher in hard wood microsites at clearcut plots because the downed wood alters the habitat more profoundly for ECM fungi in the clearcuts.

Objective 4: To explore the capacity of individual ECM fungal taxa for plasticity among microsites, and to determine if there is evidence of functional complementarity among species that co-occur in the same microsite.

Hypothesis 4: Individual ECM fungal species will show altered patterns of enzyme activity in different microsites, and co-occurring ECM fungal species will show different and complementary patterns of enzyme activity in the same microsite (Figure 1.7 and 1.8).

Prediction 4: If some ECM fungal species are capable of some phenotypic plasticity, and others are uniquely adapted to particular substrates, then I would detect altered patterns of enzyme activity by some species in different substrates, but also complementary patterns of enzyme activity between co-occurring species. Since complementarity is related to species diversity, these patterns will differ between clearcut and forest plots (Figure 1.9). *I predict that at least some ECM fungal species will show altered patterns of enzyme activity in different microsites, but that some co-occurring ECM fungal species would show different and complementary patterns of enzyme activity in the same microsite substrate, demonstrating both phenotypic plasticity, and adaptation to and competition for a specific niche. I suspect that these patterns will be different in the species-poor clearcut versus the species-rich forest.*

a)



b)

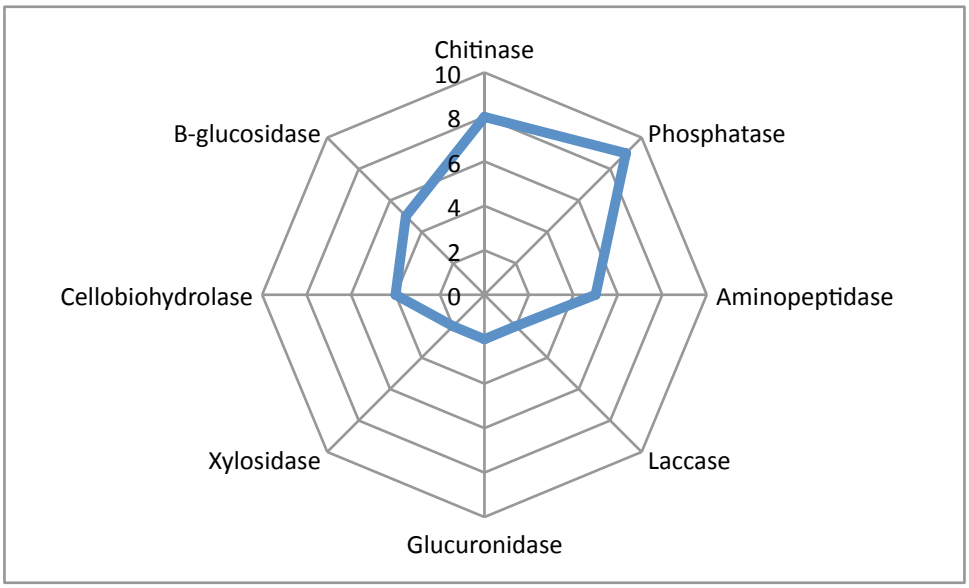


Figure 1.7 Hypothetical enzyme activity profile for species X in a) decayed wood, and b) hard downed wood microsites as predicted by Hypothesis 4.
This species will demonstrate trait plasticity for depolymerase enzymes; cellulolytic enzyme activity will be higher in decayed wood microsites (predicted to be a high carbon substrate), while N and P associated enzymes will be higher in hard downed wood microsites (predicted to contribute N and P compounds to the soil). The scale is based on hypothetical absolute values of enzyme activity rates per root surface area.

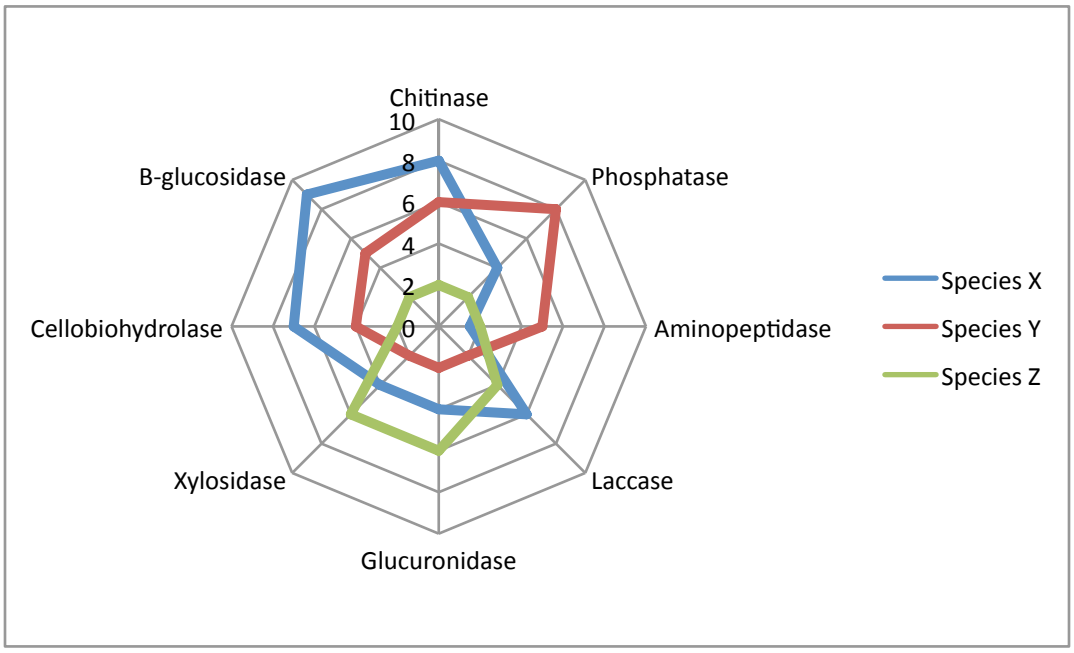


Figure 1.8 Hypothetical enzyme activity profile for species X, Y, and Z in the same microsite as predicted by Hypothesis 4.
These species will demonstrate functional complementarity for depolymerase enzymes; each species will perform uniquely in this substrate, contributing to the overall efficiency of the system. If the system is also highly diverse (i.e. species rich), there may be a high degree of functional overlap (or redundancy).

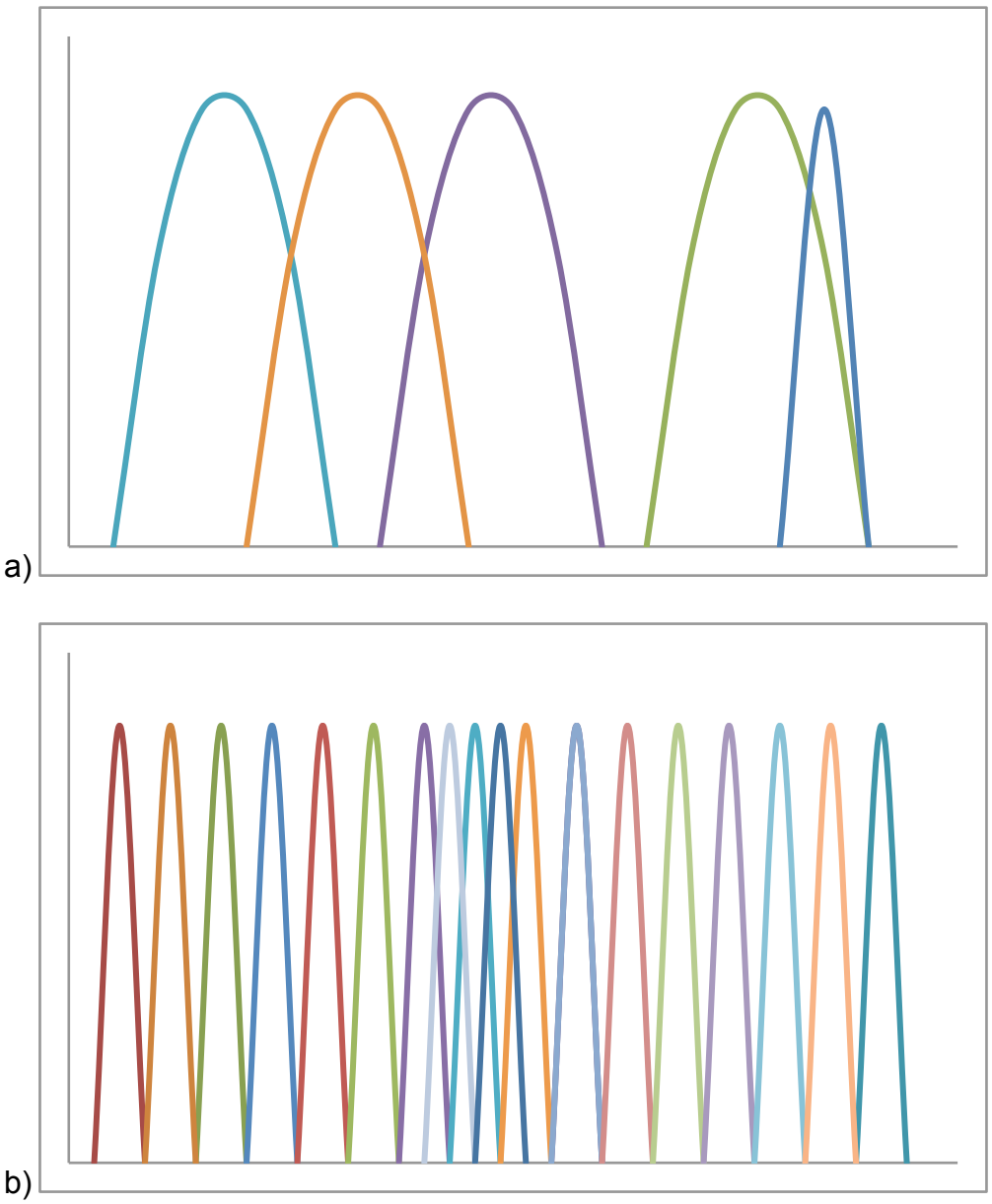


Figure 1.9 Hypothetical patterns of the functional relationship among ECM fungal species in a) clearcuts may differ from those in b) intact forests.
Clearcuts are species poor, and the identity and physiological plasticity of key taxa may be important for retaining function in this disturbed system. Forests are species rich, and many different taxa may have similar, or overlapping functions, which contribute to the resilience of this system. In both figures, each curved line represents one species and its functional contribution to the system. As species are added, the resilience of the system increases, especially when the function of some species begin to overlap. In the clearcut (a) there are few species, most with a broad range of function; in the absence of other species, their realized niche approaches their fundamental niche. The loss of one of these key species could result in loss of function in the clearcut. In the forest (b) there are many species, with narrowly defined function due to competition for niche space. This system exhibits functional complementarity and redundancy (where species overlap), and the loss of one species may not result in loss of function. These figures are modified from Botton et al. (2006).

1.3.3 The community composition and enzymatic activity of fungal hyphae colonizing decayed wood and mineral soil microsites differs among forest plots (Chapter 4).

Objective 5: To determine whether the composition and physiological activities of the fungal community in general, and the ECM community in particular, present as extramatrical hyphae in the undisturbed forest, differs among decayed wood, downed wood, and mineral soil microsites.

Hypothesis 5: Taxonomic and functional differences will be found in the overall fungal community, and in the ECM fungal community among decayed wood, mineral soil, and hard downed wood microsites.

Prediction 5: If fungal communities in forests are structured by substrate properties, and if different substrate types are colonized by the hyphae of unique fungal taxa, I would detect a shift in the fungal community among microsites of decayed wood, mineral soil, and hard downed wood. If the ECM fungal community differs among substrates, I would also detect altered patterns of ECM fungal exoenzymes activity among the microsites. *I predict that both taxonomic and functional differences will be found in the fungal hyphae community among microsites of decayed wood, mineral soil, and hard wood because the substrates differ greatly in abiotic properties, including nutrient status, and because fungal communities are strongly structured by these properties.*

2 Coarse woody debris retention in subalpine clearcuts affects the community structure of ectomycorrhizal fungi within fifteen years of harvest

2.1 Synopsis

Fallen trees and branches are important for mammals (Bunnell and Houde, 2010; Craig et al., 2006), non-vascular plants and lichens (Arsenault, 2002; Jonsson et al., 2005), and ectomycorrhizal (ECM) fungi (Harvey et al., 1979; Olsson et al., 2011; Tedersoo et al., 2003). Large pieces of hard woody debris change the abiotic properties of the soil in the short term (i.e. up to 15 years) (Bunnell and Houde, 2010), and as they decay over hundreds of years, (Bütler et al., 2007; Kayahara et al., 1996; Laiho and Prescott, 1999; Spears et al., 2003; Spears and Lajtha, 2004).

The roots of tree seedlings and their ECM fungal symbionts penetrate downed wood when it has decayed sufficiently (Christy et al., 1982; Harmon et al., 1986). Ectomycorrhizae are an integral part of most temperate forest soil ecosystems, but clearcut logging results in the loss of some ECM fungi that dominate forest communities (Dickie et al., 2009; Jones et al., 2003; Mah et al., 2001). Coarse woody debris (CWD) generated during harvesting is a remnant of the original forest (Elliott et al., 2007), and may harbour or provide habitat for forest-associated ECM fungi when it is retained on cutblocks after clearcut harvesting.

Succession in the ECM root tip community after disturbance has been observed in many forest systems (Tedersoo et al., 2003; Twieg et al., 2007). Most studies on medium to long-term succession have been done on chronosequences (i.e. they are performed at multiple sites that vary in time-since-disturbance, and are used as proxies for how the system might change if it were possible to repeat the experiment at the same site over long time scales) (Twieg et al., 2007 and references therein). The Sicamous Creek Silvicultural Systems Trial was established in 1994 in the Englemann Spruce - Subalpine Fir (ESSF) biogeoclimatic zone (Vyse, 1997; Lloyd et al., 1990). It provides an ideal opportunity to study succession after clearcut logging on one site. Replicated plots with varying amounts of CWD were created in 10 ha clearcuts during the harvesting. This provides an opportunity to observe how the ECM community is affected by post-harvest site manipulation.

The first objective of our study was to test how the retention of CWD on clearcut blocks contributes to ECM fungal community structure in regenerating stands in the medium term (i.e. less than15 years post-harvest). The second objective of our study was to observe how the ECM community had changed since experiments undertaken immediately after harvesting (for example Hagerman et al., 1999, Jones et al., 2002). We identified ECM fungal communities by Sanger sequencing of fungal DNA from ectomycorrhizae, and pyrosequencing of DNA from ECM fungal hyphae. This allowed us to compare communities between the treatment plots, to examine how the ECM community had changed over time,

and led us to further investigate one dominant fungal taxon, *Alloclavaria purpurea* (Fries), which is not known to be mycorrhizal. To provide insight into its trophic status, we used isotope analysis of its sporocarps.

Hypothesis 1: Taxonomic differences will be not be found among ectomycorrhizae on sapling root systems between CWD treatments nor among ECM hyphae in sapling root zones between CWD treatments.

Hypothesis 2: Succession in the ECM fungal community has occurred since the initial studies at this site.

2.2 Methods

2.2.1 Site description and experimental design

The experimental area that is the focus of this chapter includes three replicate 10 ha clearcut blocks designated A, B, and C. Cutblock A is northwest-facing and ranges in elevation from 1583 m to 1622 m, B is north-facing and ranges from 1648 m to 1678 m, and C is west-facing and ranges from 1739 m to 1769 m. In each cutblock, two 1 ha treatment plots were established in the summer of 1995: one where coarse woody debris (CWD) generated during the harvesting was retained (CWD+) and one where as much coarse woody debris as possible was removed (CWD-) (Table 2.1).

Table 2.1 Coarse woody debris volume on 1 ha retention and 1 ha removal plots in all 10 ha blocks at Sicamous Creek.

Block	Retention (mm^3 ha^{-1})	Removal (mm^3 ha^{-1})
A	453.3[1]	57.0
B	347.9	112.9
C	416.9	60.4

[1]CWD volumes are from Craig et al., 2006.

2.2.2 Root tip sampling and molecular identification of fungi from ectomycorrhizae

One long lateral root was sampled from each of ten 10 yr-old spruce saplings in each plot (2 CWD treatments X 3 blocks X 10 saplings = 60 root samples) in mid September 2006. This was accomplished by isolating, and digging out attached roots that were approximately 1 m long. Roots were rinsed gently in tap water and cut into 1 cm segments. The segments were picked at random from a grid and the root tips examined under 100X magnification, until 100 (or all) live ectomycorrhizal root tips per sample had been examined. Turgid tips with a fungal mantle and/or Hartig net were considered ectomycorrhizal and were morphotyped based on Agerer's (1987-2002) descriptions and the instructions of Goodman et al. (1996). Morphotypes were distinguished by the type of branching, colour, texture, abundance of hyphae, presence of rhizomorphs, and other microscopic features of the mantle and emanating hyphae. Two tips from each morphotype from every sapling root sample were stored at -80 °C for DNA extraction.

DNA was extracted directly from at least one root tip per pair by grinding with ceramic beads (Qiagen DNEasy kit, Qiagen Inc. Mississauga, ON). The internal transcribed spacer (ITS) region of fungal ribosomal DNA was amplified using forward primer NSI1 (5'-GATTGAATGGCTTAGTGAGG-3') and reverse primer NLC2 (5'-GAGCTGCATTCCCAAACAACTC-3') (Martin and Rygiewicz, 2005). This primer set is designed to amplify both ascomycete and basidiomycete DNA, but the basidiomycete-specific primer pair ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4B (5'-CAGGAGACTTGTACACGGTCCAG-3') (Gardes and Bruns, 1993) was used if the first primer pair failed a second PCR reaction with template DNA diluted 1:10. Each 30 µl PCR reaction mixture included 3.0 µl 10X buffer, 0.6 µl 10 mM dNTPs, 0.36 µl 10 mg ml^{-1} BSA, 2.76 µl 0.1 M $MgCl_2$, 0.14 µl of each forward and reverse primer, 0.75 U *Taq* polymerase and 1.0 µl of template DNA. Thermocycler conditions were 95 °C for 10 min, followed by 34 cycles of: 94 °C for 45 s, 54 °C for 45 s, 72 °C for 1 min, and finally 72 °C for 10 min followed by cooling to 4 °C. PCR products producing single bands of expected size on 1 % agarose gels were cleaned of excess primers and free nucleotides (ExoSAP-IT, USB Corp, Ohio, U.S.A.). Amplicons were sequenced with forward primer ITS1F and reverse primer ITS4 using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems Inc, Foster City, CA, U.S.A.).

2.2.3 Sampling and molecular identification of fungal hyphae within mesh bags

Three 5 x 10 cm, 50 µm mesh bags filled with 30 g of silica sand were buried among the roots of ten 10 yr-old spruce saplings at every plot in mid September 2006. The saplings chosen were immediately adjacent to those from which root tips had been collected. The bags were placed in three equidistant cardinal directions around the stem of the saplings so that they lay immediately adjacent to a lateral root and were sandwiched between mineral and organic soil layers. Such bags are highly effective in selecting for ECM hyphae because the 50 µm mesh excludes roots, and the absence of a carbon source in the substrate minimizes colonization by saprotrophic fungi (Anderson and Cairney, 2007; Kjøller, 2006; Korkama et al., 2007; Wallander et al., 2001). We recovered the mesh bags from late August through late September 2007. Each bag was kept cool until processing, which occurred within four days of collection.

Bags were opened and examined for presence of mycelia and aggregation of sand particles under a dissecting microscope and ranked according to a modified scale of Wallander et al. (2004): 0. No mycelia, 1. Occasional mycelia, 2. Sparse mycelia with or without aggregation of sand particles, 3. Plenty of mycelia with or without aggregation of sand particles, and 4. Plenty of mycelia with plenty of aggregated sand particles. The sand from the three mesh bags per sapling was then combined and mixed thoroughly. Approximately 5 g was taken for molecular

analysis and stored at -80 °C. The few mesh bags with holes, split seams or root infiltration were not combined with the others nor used for further analysis.

DNA was extracted from1 g sub-samples from each combined sample with the MoBio Ultra Clean Soil DNA Extraction Kit using the Alternative Protocol for maximum yields (MoBio Laboratories Inc., Carlsbad CA, U.S.A). DNA concentrations were low (@5-15 ng μl^{-1}) compared to predicted kit outcome, but an increase in the proportion of template DNA added to the PCR mixture resulted in good success with excellent quality amplification of the fungal ITS1 region from nine of ten samples in each plot. A unique pyrosequencing primer was used for each of the six plots. Every 50 µl PCR reaction mixture included 5.0 µl 10X buffer, 1.0 µl 10 mM dNTPs, 2.0 µl 50 mM $MgCl_2$, 1.0 µl 10 µM of each forward and reverse fusion primer, 1 U Platinum *Taq* polymerase (Invitrogen Corp, Carlsbad CA, U.S.A.) and 1.0 to 2.0 µl of template DNA (for a final concentration of 0.2 ng μl^{-1}). The forward fusion primer used was 5'-CCATCTCATCCCTGCGTGTCTCTCCGACTCAG (Titanium A Primer) XXXXXXXXXX CTTGGTCATTTAGAGGAAGTAA (ITS1F)-3'(Gardes and Bruns, 1993), where 'XXX…" represents one of six multiplex identifier (MID) tags. The six MID tags were CGAGAGATAC, ATACGACGTA, TCTACGTAGC, TACTCTCGAG, TCGTCGCTCG, and ACGCGAGTAT. The reverse primer for all reactions was 5'-CCTATCCCCTGTGTGCCTTGGAGTCTCAG (Titanium B Primer) GCTGCGTTCTTCATCGATGC (ITS2)-3'(White et al., 1990). Thermocycler conditions were 94 °C for 2 min, followed by 35 cycles of 94 °C for

30 s, 55 °C for 30 s, 68 °C for 1 min, and a final extension step of 68 °C for 10 min. Good quality single bands on 1 % agarose gels were cleaned with Agencourt Ampure XP magnetic bead PCR purification system (Beckman-Coulter, Danvers MA, U.S.A.), checked again for band quality and the removal of primer dimers and other low molecular weight product on new agarose gels, and quantified against a low mass DNA ladder and with a NanoDrop micro-volume spectrophotometer (Thermo Scientific, Wilmington, DE. U.S.A.).

Every sample from each plot was amplified individually with a primer tag unique to that plot, and all amplicons (nine successful samples from each plot) were pooled in an equimolar mixture by combining equal amounts of each PCR amplicon at a standard concentration. The final 20 ng ul^{-1} mixture, containing six unique pyrosequencing primers, each representing one of six different plots, was amplified in a 1/8 plate Next Generation pyrosequencing reaction on a Roche GS-FLX at the McGill University and Genome Quebec Innovation Center.

2.2.4 Sequence processing and phylogenetic-based naming

Sequence contigs of fungal DNA from ectomycorrhizae were aligned and corrected using Sequencher 4.2 (Gene Codes Corp, Ann Arbor, MI, U.S.A.), and primer removal was accomplished with MOTHUR v. 1.16.0 (Schloss et al., 2009). The entire ITS region was isolated using the Fungal ITS Extractor (Nilsson et al., 2010) and compared against the GenBank database (BLASTn, Altschul et al., 1997) via the ITS Pipeline (Nilsson et al., 2009). The ITS sequences were also

aligned with MAFFT v. 5 (Katoh et al., 2002) and clustered in MOTHUR at 95% similarity. A representative from each operational taxonomic unit (OTU) was imported into MEGAN v. 4.40.1 (Huson et al., 2007) for aid in taxonomic placement.

Root tip OTUs were assigned a species name if the sequence had 97 % similarity over at least 450 bp to a vouchered database match, a genus name if 94-96 % similarity, a family name if 91-93 %, and an order, class or division name if < 90 %. Discretion was used for sequences approaching 600 bp matches and for those with fewer than 450; in the former case, a lower % match was considered acceptable for naming, and in the latter, a higher % match was required. Species names were rarely assigned to sequence matches of fewer than 300 bp. Mesh bag OTUs derived from short pyrosequencing reads were placed in a taxon no smaller than genus, based on assignment by MEGAN, with the exception of some dominant taxa known to be present in the high quality root tip sequences.

Pyrosequencing data from hyphae in the mesh bags were imported into MOTHUR, primers were removed, and sequences were trimmed (min 100 bp, max 400 bp, pdiffs =1, maxambig = 0, maxhomop = 8). Examination of the list of eliminated sequences showed that the latter filter was strongly biased against members of the Pyronemataceae; consequently, sequences eliminated by this filter were retrieved and used in all further analyses. The Fungal ITS Extractor

was used to isolate the ITS1 region, and these sequences were run through the ITS Pipeline for matches to the GenBank database with and without uncultured fungi. The ITS1 sequences were aligned with MAFFT, and assembled into distance matrixes in MOTHUR (countends =F, cutoff=0.10). OTUs were clustered from 90 to 99 % in order to define a sequence similarity cutoff where accumulation curves did not reach an asymptote yet did not increase exponentially. One randomly chosen representative sequence from each OTU was imported in MEGAN for taxonomic placement (based on lowest common ancestor (LCA) parameters: min support = 1, minimum score = 200, top percent = 10, disable = environmental samples). Upon closer inspection several hyphal sequences that had originally been excluded because they occurred as singletons clustered with positively-identified, high-quality sequences of fungi sampled from root tips on the same plots. For analysis of the ECM hyphal community, we included singleton ECM OTUs that could be identified to at least the family level.

2.2.5 Analysis of ECM community structure

Rarefied ECM root tip taxon richness (observed and estimated), diversity and evenness were calculated in EstimateS (V 8.2) (Colwell, 2009). Many root samples had fewer than 100 mycorrhizal root tips, and hence sample sizes varied. Therefore, sample-based rarefaction (without replacement) was applied in order to correct richness estimates for the unequal number of tips per sample. Sobs (Mau Tau) best illustrated observed taxon richness (based on the taxa we

actually detected), while Chao 2 (classic) and Jackknife 2 were chosen to estimate richness since they rely on rare species (singletons and doubletons) which were expected to form a large component of our dataset (Chao, 2005). Simpson's reciprocal diversity index was used to calculate taxon evenness. Taxon richness by sapling and by plot fit a normal distribution, and homogeneity of variance was confirmed using a Bartlett's test. Consequently, treatment effects on rarefied richness, diversity, and evenness at the sapling level (e.g. mean number of taxa among root samples per plot) were examined using a mixed-effect hierarchical ANOVA with treatment nested in plot (n=10 per plot) (Statistica v. 6.1; StatSoft Inc., 2003). Estimated richness as bias-corrected Chao1, and Jacknife1 or Jacknife2 was calculated in MOTHUR for total fungal OTUs from mesh bags per plot. Observed ECM fungal richness from mesh bags per plot was also calculated. All effects at the plot level (n=3) were tested with one-way ANOVA (Statistica v. 6.1; StatSoft Inc., 2003).

Detrended Correspondence Analysis (DCA) and Nonmetric Multidimensional Scaling (NMS) in PCORD v. 5.0 (McCune and Mefford, 1999) were used to visualize root tip and mesh bag community data using both presence-absence and relative abundance of ECM taxa. The root tip relative abundance matrix (% of total tips) was calculated by dividing the number of tips from each taxon per sapling by the total number of tips counted on that sapling (e.g. 4 tips/100 tips). Given that pyrosequencing read abundance can be a misleading method of comparing the relative abundance of different taxa (Amend et al., 2010),

statistical analyses of ECM hyphal community data relied on presence or absence only. DCA ordinates species and sample units by rescaling axes in order to minimize within-sample variance; it uses a chi-squared distance measure and can result in misleading solutions for complex datasets with a large proportion of rare species but is suitable for analyses of ECM community structure (Baier et al., 2006; Izzo et al., 2005; Tedersoo et al., 2008). NMS and permutational ANOVA are all especially well suited to non-normal datasets like ours that contain many zeroes (McCune and Grace, 2002). Differences in taxon occurrence and relative abundance between treatments were tested statistically using permutational multivariate ANOVA (Anderson, 2005) and the default Bray-Curtis distances. For sapling-level analyses, the analysis was hierarchical, with treatment nested in block, n=10 observations per plot. For plot-level analyses, a one-way permutational MANOVA was conducted (n=3). For all analyses of ECM fungal community structure, statistically significant results were acceptable at $p \leq 0.10$.

2.2.6 Frequency and abundance of individual ECM fungal species

Differences between treatments for plot-level frequency and relative abundance of key ECM species on root tips, and for plot-level relative abundance of key ECM species in mesh bags was tested for each species individually with one-way univariate ANOVA (n=3). To calculate plot-level frequency, we counted the number of saplings (out of 10) for each plot on which a species was found and divided this by the number of occurrences of all taxa per plot (= relativized by

sample and by plot) so that plots could be compared (n=3). ECM taxa were defined as key species for comparison because they were the most abundant in terms of number of root tips or pyrosequencing reads (by more than one order of magnitude over other taxa) and because they were dominant in both communities. Calculating the relative abundance of reads was appropriate for the ECM hyphae community in this case, since the objective was to explore within-species variation only (Amend et al., 2010). Indicator Species Analysis as defined by Dufrêne and Legendre (1997) was used in PC-ORD to further explore the contribution of individual ECM species to CWD retention and removal treatments.

2.2.7 Testing the trophic status of *Alloclavaria purpurea*

DNA of *Alloclavaria purpurea* was amplified frequently from ectomycorrhizae. We cloned the amplified DNA to test whether DNA of known ECM fungi were also present in the samples since *A. purpurea* has not been reported as an ECM fungus. We hypothesized that *A. purpurea* could be present as a saprotroph in the rhizosphere. The detection of only DNA of *A. purpurea* in the cloned samples would support its status as ectomycorrhizal. Fragments of interest for cloning were ligated into a vector and then transformed into competent (*E. coli*) cells using a TOPO TA Cloning Kit following the manufacturer's recommendations (Invitrogen Corp, Carlsbad CA, U.S.A.) (Landeweert et al., 2003; Lindahl et al., 2007). These cells were plated out at two dilutions; 16 individual colonies grown from bacteria that successfully took up the fragment of interest were chosen from

each sample for a second round of PCR amplification (using the original primers) and sequencing.

In September 2010 sporocarps of both saprotrophic (*Auricularia auricula-judae* (2), *Galerina marginata* (2), *Lycoperdon rimulatum* (1), *Mycena tenax* (2), *Pholiota lubrica* (2), *Pluteus pouzarianus* (1)) and ECM fungi (*Clavariadelphus subfastigiatus* (3), *Cortinarius camphorus* (1), *C*. cf. *alboviolaceus* (1), *C. caperatus* (3), *C. junghnii* (1), *Hygrophorus eburneus* (3), *Laccaria laccata* (2), *Lactarius deliciousus* var. *deterrimus* (4), *Russula* aff. *curtipes* (3), *R*. *queletii* (2), *Sarcodon imbricatus* (3)) were collected from the forest adjacent to Cutblock B, where we observed fruiting bodies of *A. purpurea*. One to four sporocarps of each species, collected at least 1 m apart, were oven-dried, ground in a ball mill, and weighed into tin capsules. Samples were analyzed for $\delta^{15}N$, $\delta^{13}C$, %N and %C by continuous flow with a Costech 4010 element analyzer (Costech Analytical Technologies Inc., Valencia CA, U.S.A.) and a Finnigan DELTAplus XP mass spectrometer (Thermo Fisher Scientific, Waltham MA, U.S.A.) at the University of New Hampshire Stable Isotope Laboratory. For each analysis, all the nitrogen and carbon isotope data were reported in δ (Delta) notation with reference to this equation: $\delta^{15}N$ or $\delta^{13}C$ = (R sample/R standard)-1) • 1000, where R = $^{15}N/^{14}N$ or $^{13}C/^{12}C$ of the sample (Mayor et al., 2009). The standard for carbon was Vienna Pee Dee Belemnite and the standard for nitrogen was atmospheric air. Laboratory standards for isotope analysis were NIST 1515 (apple leaves), NIST 1575a (pine needles) and tuna muscle, and *Boletus* tissue.

Ten percent of samples were analyzed in duplicate as blind internal quality controls.

Fungal DNA was extracted and amplified using fungal specific primers (ITS1F and ITS4) from frozen samples of sterile cap tissue of each sporocarp with a Sigma Extract-N-Amp Plant PCR Kit (Sigma Aldrich, St. Louis MO, U.S.A.) according to manufacturer's instructions. Thermal cycler conditions for 50 µl reactions were: a 3 min initial denaturation at 94 °C, followed by 35-40 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, and a final 10 min extension at 72 °C. Amplicons were visualized on a 1 % agarose gel, cleaned with Mag-Bind™ E-Z Pure magnetic beads according to the 96-plate protocol (Omega Bio-tek, Norcross GA, U.S.A.), and quantified with a NanoDrop micro-volume spectrophotometer (Thermo Scientific, Wilmington DE, U.S.A.) prior to in-house Sanger sequencing as above.

One-way ANOVAs were performed to test for differences between saprotrophic and ECM sporocarps (excluding *A. purpurea*) in $\delta^{15}N$ and $\delta^{13}C$ signatures after confirming the assumptions of normality and homogeneity of variance. The relationship of the isotopic signatures of *A. purpurea* was then compared to those of known trophic status in two ways. First, 95% confidence limits were drawn around data points of saprotrophic and ECM sporocarps on a scatter plot of $\delta^{15}N$ vs. ^{13}C. Data points of the four *A. purpurea* sporocarps were then added. Second, the $\delta^{15}N$ and $\delta^{13}C$ data from known saprotrophic and EMF sporocarps

were supplied to a discriminant analysis function to calculate a rubric for defining the two groups by isotopic signatures as per Mayor et al. (2009). Discriminant function analysis essentially uses data from members of pre-determined groups to generate a linear combination of the variables that maximizes the likelihood of categorizing the supplied data correctly. This can then be used to categorize data from unknown subjects into the pre-determined groups (Quinn and Keough, 2002). The rubric was used to classify the four *A. purpurea* samples after testing its efficiency in assigning the original data into the appropriate trophic group. All analyses were run on JMP IN 4.0.2.

2.3 Results

2.3.1 ECM fungal communities on root tips

The 197 fungal ITS sequences resulting from PCR and Sanger sequencing of ECM root tips were distributed among 89 OTUs; 38 of these could not be named beyond the level of phylum, and five were identified as saprotrophs. The remaining 46 ECM taxa, representing 69% of the tips collected, were used for analysis. A Coleman rarefaction curve (Figure A.1) suggested that identifying the remaining unnamed OTUs would not have contributed greatly to the detection of new taxa. Morphotype data were relied upon exclusively for detecting the presence of *Cenococcum geophilum*. ITS sequences generated from these mycorrhizae resolved only to Dothideomyceta. When pooled across all samples, the most abundant ECM taxa encountered on root tips were *Thelephora* spp. (primarily *T. terrestris*), *A. purpurea* (including Agaricomycetes 1, likely *A.*

purpurea), *Amphinema* spp. (primarily *A. byssoides*), and *Tylospora* spp. (primarily *T. asterophora*) (Table 2.2). All tips that were very likely matches were included, and proportions were calculated by dividing the number of ECM root tips per taxon by the number of all root tips named as listed in Table A.1. Known saprotrophic fungi detected on root tips included members of the Basidiomycota: *Nolanea* sp. (Entolomatacea, Agaricales, (Hymenomycetes) Agaricomycetes), *Mycena* spp. (Mycenaceae, Agaricales, Basidiomycetes), and *Galerina* sp. (Hymenogastraceae, Agaricales, Agaricomycetes), plus one ascomycetous member of the Hyaloscyphaceae (Helotiales, Leotiomycetes).

Table 2.2 The relative abundance of ECM fungal taxa encountered on sapling root tips when pooled across all samples. Proportions were calculated by dividing the number of ECM root tips per taxon by the number of all root tips named.

Taxon	Relative abundance (%)
Thelephora spp.[1]	26.5
Alloclavaria purpurea	21.1
Amphinema spp.	9.5
Tylospora spp.	8.5
Inocybe spp.	7.5
Lactarius spp.	4.7
Pyronemataceae 1-6[2]	4.3
Russula spp.	1.9
Cortinarius/Dermocybe spp.	1.2
Cenococcum geophilum	1.2
Hygrophorus sp.	1.1
Ceratobasidiaceae	1.1
Atheliaceae 2[3]	0.4
Entoloma sp.	0.4
Sebacina sp.	0.3
Atheliaceae 1[4]	0.2
Hydnaceae	0.05

[1]Spp. implies that more than one OTU was used to calculate relative abundance
[2]Likely *Wilcoxina mikolae* [3]Possibly *Amphinema diadema* [4]Primarily *Piloderma* spp.

2.3.2 ECM fungal communities occurring as extramatrical hyphae

Pyrosequencing analysis of DNA extracted from fungal hyphae trapped in mesh bags generated 121,962 reads; quality control, which included all trimming and filtering described in the methods, reduced this number to 87,620 ITS1 sequences for analysis. Rarefaction curves generated in MOTHUR supported 95% sequence similarity as the cutoff for clustering of hyphal OTUs; using 95% similarity resulted in 5347 OTUs, including singletons (refer to Methods) (Figure A.2). Curves based on 90-94% similarity appeared to reach asymptotes, which would be unexpected for samples of soil fungi, while curves generated using percentage similarities of 96-99% began to rise rapidly. When a representative sequence from each of the OTUs was positioned taxonomically by MEGAN, only 45.0% were placed into taxa of phylum or lower classification: 430 OTUs at phylum through order, and 1972 OTUs at family through species. Fifty ECM taxa were identified to at least family level, and these taxa were used for subsequent analysis. Many short pyrosequencing reads that were initially resolved to genus by MEGAN clustered at 95% with excellent quality fungal sequences from root tips. These were elevated to the species they matched for all subsequent analyses.

There were only 120 OTUs with greater than 100 reads, and although these comprised only 2.2% of all OTUs, they represented 63.8% of the reads (Table A.2). OTUs with the largest number of reads from mesh bags in clearcuts at

Sicamous Creek were ECM taxa. *Amphinema* spp. (primarily *A. byssoides*) dominated the fungal community trapped in mesh bags, based on read abundance, with 39.1 % of reads (all OTUs that are very likely matches are combined, and proportions are calculated by dividing the number of ECM reads represented by the total number of reads listed in Table A.2). *Thelephora* spp. (primarily *Thelephora terrestris*) with 17.0 % of reads, *Wilcoxina mikolae* (including likely members of the Pyronemataceae) with 6.8 % of reads, and *Tylospora* spp. (primarily *Tylospora asterophora*) with 4.6 % of reads, also appear to be major members of the extramatrical hyphae community. The remaining ECM taxa represented by OTUs containing more than 100 reads include: *Laccaria laccata* (0.6 %), *Pseudotomentella tristis* (0.3 %), *Inocybe jacobi* (0.3 %), *Entoloma* sp. (0.2 %), *Sebacina vermifera* (0.2 %). Saprobes commonly detected in the mesh bags included *Cryptococcus* sp. (Tremellales, Agaricomycotina, Basidiomycota), *Mortierella* sp., and other members of the Mortierellales (Mucormycotina, Zygomycota), plus members of the Strophariaceae: *Hypholoma* sp., *Pholiota* sp. and *Psilocybe montana* (all Agaricalean Basidiomycota). Numerous mitosporic Helotiales were detected, as well as the Eurotiomycetes *Calyptrozyma arxii* and *Cladophialophora* sp. (Pezizomycotina), and other pathogenic Ascomycota (e.g. *Leptodontidium* sp. and *Physalospora scirpi*).

2.3.3 Effects of CWD retention of the structure of ECM fungal communities

Retention of CWD did not appear to affect indices of ECM fungal community diversity on colonized root tips. Mean sapling-level taxon richness and total rarefied plot-level richness were almost identical between CWD treatments (Table 2.3). Furthermore, plot-level ECM fungal diversity and estimated total richness based on Chao 2 or Jacknife 2 estimators did not differ.

Table 2.3 ECM root tip and hyphae taxon richness, diversity, and evenness.

Plot	Retention[1]	Removal[1]	P-value
Total root tips represented	1903	1835	Overall = 3738 tips
Mean number of root tip taxa per sapling	2.3 (1.0)	2.1 (1.2)	0.4
Rarefied number of root tip taxa per plot (S)	13.0 (4.6)	13.0 (1.0)	1.0
Number of root tip taxa estimated per plot (Chao 2)	28.7 (19.2)	46.5 (23.9)	0.4
Number of root tip taxa estimated per plot (Jack 2)	25.9 (12.3)	29.1 (5.1)	0.2
Root tip Simpson diversity per plot (D)	5.9 (4.8)	4.7 (2.1)	0.7
Root tip Simpson evenness per plot E = D/S	0.41 (0.19)	0.36 (0.17)	0.8
Number of ECM hyphae taxa per plot	28.0 (1.7)	27.0 (9.6)	0.9

[1]All values after plot totals are SD.

Similarly, we detected no effect of the retention of CWD on fungal community diversity in the mesh bags. Specifically, when OTUs (including singletons) representing the entire fungal community were included, observed or estimated OTU richness, Simpson diversity, or evenness were not affected by retention of

CWD (data not shown; $p>0.5$). Mean plot-level richness was also very similar between CWD retention and removal plots when only ECM taxa from the mesh bags were considered (Table 2.3).

By contrast, species composition of the ECM fungi on root tips of saplings had responded to 11 years of CWD retention. Permutational MANOVAs detected differences in sapling-level ECM communities, based on relative abundance and presence-absence of fungal OTUs, with significant block effects (Table 2.4). Ectomycorrhizal fungal root tip communities at the plot level were not affected by retention of CWD. A DCA ordination of ECM root tip relative abundance (with no downweighting of rare species) gave a three dimensional graph, of which axes 1 and 2 explained most of the variation (57.9 %) (Figure 2.1.) All retention plots separated from two of three removal plots along axis 2 (32.5 %). One removal plot remained aligned with the retention plots because of its association with *T. terrestris.* An NMS ordination of ECM root tip presence-absence data (Figure 2.2a) gave a 2D solution with excellent final stress (0.004); axis 1 represents 63.8 % of the variation, while axis 2 represents an additional 13.9 % for a total of 77.7 %. The dominant species at this site, in terms of occurrence, occupy the center of the ordination, while other taxa are more closely aligned to one sample plot.

Table 2.4 Permutational MANOVAs comparing sapling-level ECM root tip communities using a) relative abundance and b) presence-absence data. N=10 saplings per CWD treatment plot.

a)

	Degrees of freedom	Sums of squares	Mean squares	F-statistic	Permutational p-value
Block	2	19278.9	9639.5	2.41	**0.002**
CWD treatment (Block)	3	17704.1	5901.4	1.48	**0.05**
Residual	54	215610.9	3992.8		
Total	59	252593.9			

b)

	Degrees of freedom	Sums of squares	Mean squares	F-statistic	Permutational p-value
Block	2	18508.9	9254.5	2.45	**0.003**
CWD treatment (Block)	3	16724.7	5578.2	1.47	**0.07**
Residual	54	204379.0	3784.8		
Total	59	239622.6			

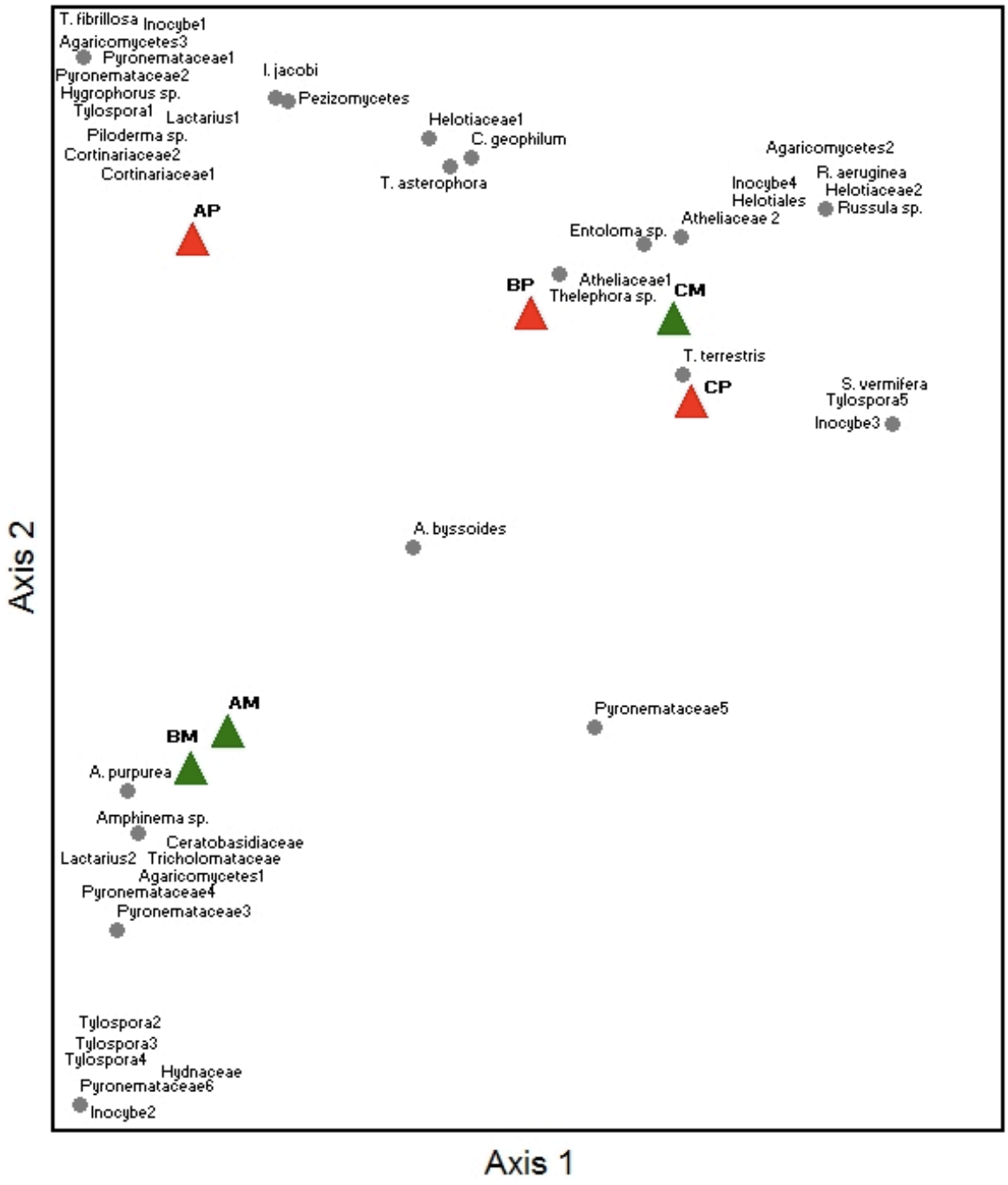


Figure 2.1 DCA ordination of ECM root tip relative abundance between CWD retention (red triangles) and CWD removal (green triangles) plots: all ECM root tip taxa are represented (solid dots).
A, B, and C refer to blocks, while P and M refer to retention (Plus) and removal (Minus) respectively. Axis 1 explains 25.4 % of the variation and Axis 2 32.5 %.

The assemblage of ECM fungi sampled as hyphae did not differ between retention and removal plots (p=0.6) at the plot scale. An NMS ordination of ECM hyphae presence-absence data (Figure 2.2b) gave a 2D solution with excellent final stress (0.023); axis 1 represented 74.1 % of the variation, while axis 2 represented an additional 13.6 % for a total of 87.7 %. Although the main ECM hyphae at this site occupy the center of the ordination, most taxa are shared across plots and therefore also cluster in the center. Analysis in MOTHUR of all fungal OTUs revealed that each plot had as many or more unique OTUs, as they shared with other plots. Of the 5437 OTUs detected, 1927 were found only in retention plots, 1831 were restricted to removal plots, and 1589 (29.7%) were shared between treatments. The 1589 shared OTUs represented 86.2% of the total reads from the samples, hence the unique OTUs may represent rare taxa.

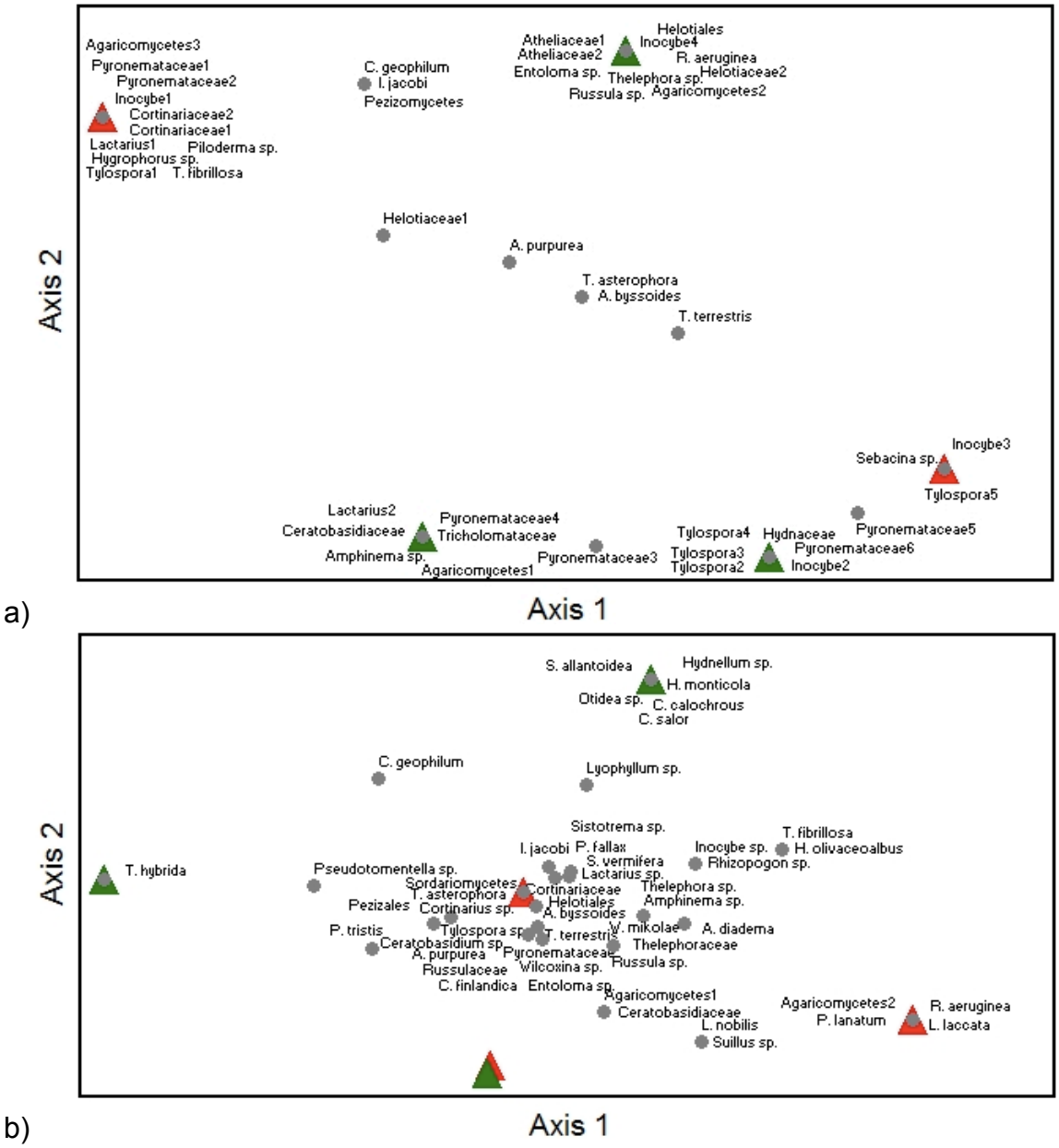
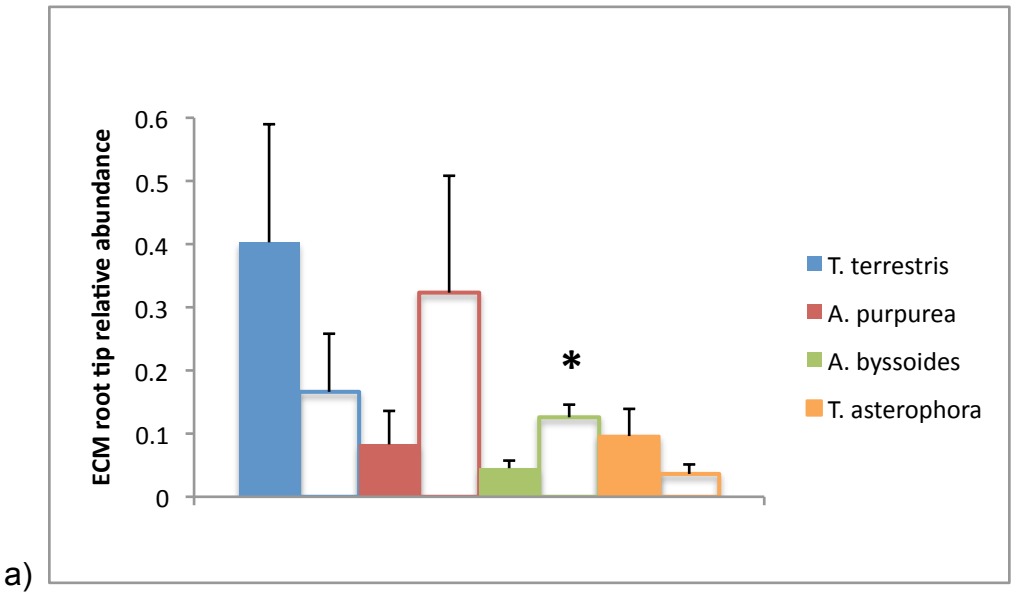


Figure 2.2 NMS ordination of a) ECM root tip and b) ECM hyphae occurrence (presence-absence) in two dimensions.
Stress is excellent for both solutions; 77.7% of the variation is explained in (a) (63.8 % by axis 1), while 87.7% is explained in (b) (74.1 % by axis 1). Red triangles represent retention plots and green triangles removal plots. In (a), the icon for one retention plot is completely hidden by the icon for one removal plot.

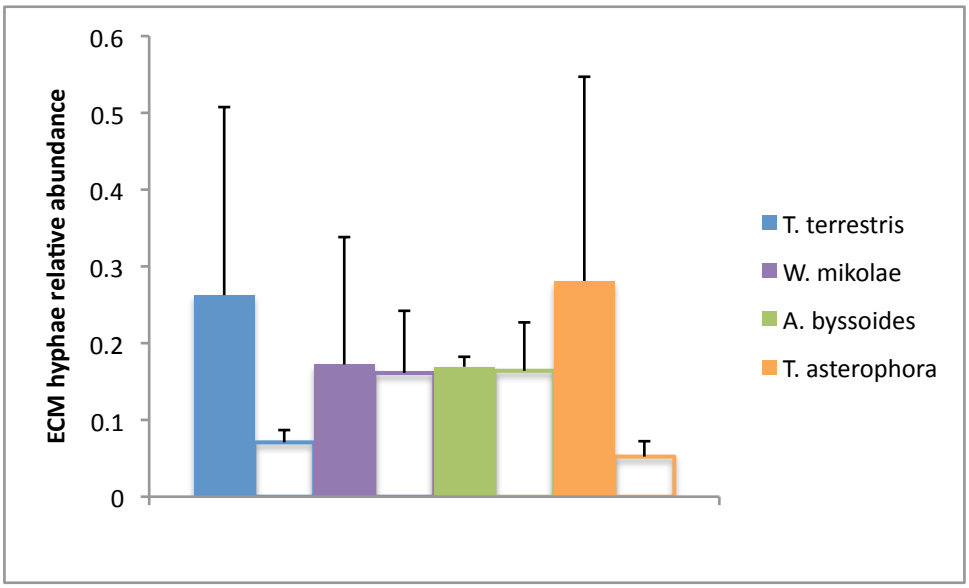
2.3.4 Comparison of ECM fungi occurring on roots and as hyphae in mesh bags

All ECM fungi detected on roots were also found as hyphae; the reverse was also true for most taxa. The few ECM taxa identified as hyphae from mesh bags but not detected on roots included *W. mikolae*, *Otidea* spp. *Pseudotomentella tristis*, *Hydnellum* spp., *L. laccata*, and *Lyophyllum* spp. (Figure A.3 a-c). *Suillus* spp. and *Rhizopogon* spp. were also detected in mesh bags, but are not shown. *W. mikolae* and *Otidea* may have been represented in one of the large root tip taxa classified as Pyronemataceae (the former very likely so) (Figure A.3a), while *Pseudotomentella tristis* and *Hydnellum* on root tips may have been classified only at the order level (Thelephorales) (Figure A.3b).

Amphinema byssoides mycorrhizae were relatively more abundant in samples from CWD removal than retention plots based on a one-way univariate ANOVA ($p=0.027$; Figure 2.3a). Indicator Species Analysis suggested that root tips formed by *A. purpurea* were indicative of removal plots ($p=0.056$) but confirmed that *T. terrestris* root tips were not an indicator of CWD retention plots ($p=0.16$). None of the ECM hyphae from dominant species showed a statistically significant affinity to either treatment plot (Figure 2.3b).



a)



b)

Figure 2.3 Relative abundance of major ECM fungal species found a) on root tips and b) as hyphae in mesh bags at retention (solid columns) and removal (open columns) plots. Bars = means + 1 standard error, N= 3 plots, * = $p < 0.05$ for difference between CWD treatments.

2.3.5 Trophic status of *Alloclavaria purpurea*

Cloning of fungal DNA from mycorrhizae producing *A. purpurea* sequences supported the contention that *A. purpurea* is a mycorrhizal fungus. When the amplified fungal DNA was cloned, only DNA of *A. purpurea* was detected. There was no evidence of a second fungus that could have formed the mycorrhiza.

Isotopic data were somewhat more equivocal. Although the $\delta^{15}N$ and $\delta^{13}C$ signatures differed ($p < 0.001$) between sporocarps of known ECM and saprotrophic fungi, and *A. purpurea* sporocarps fell within the 95% confidence interval of the ECM fungi (Figure 2.4.), Discriminant Analysis categorized three of the four *A. purpurea* sporocarps as saprotrophic. However, when the linear functions generated by Discriminant Analysis were reapplied to the original data used to generate the functions, they were not able to perfectly predict the trophic status of known sporocarps. They correctly classified all 10 saprotrophic sporocarps, but incorrectly classified two of 26 known ECM sporocarps as saprotrophic (Figure 2.4).

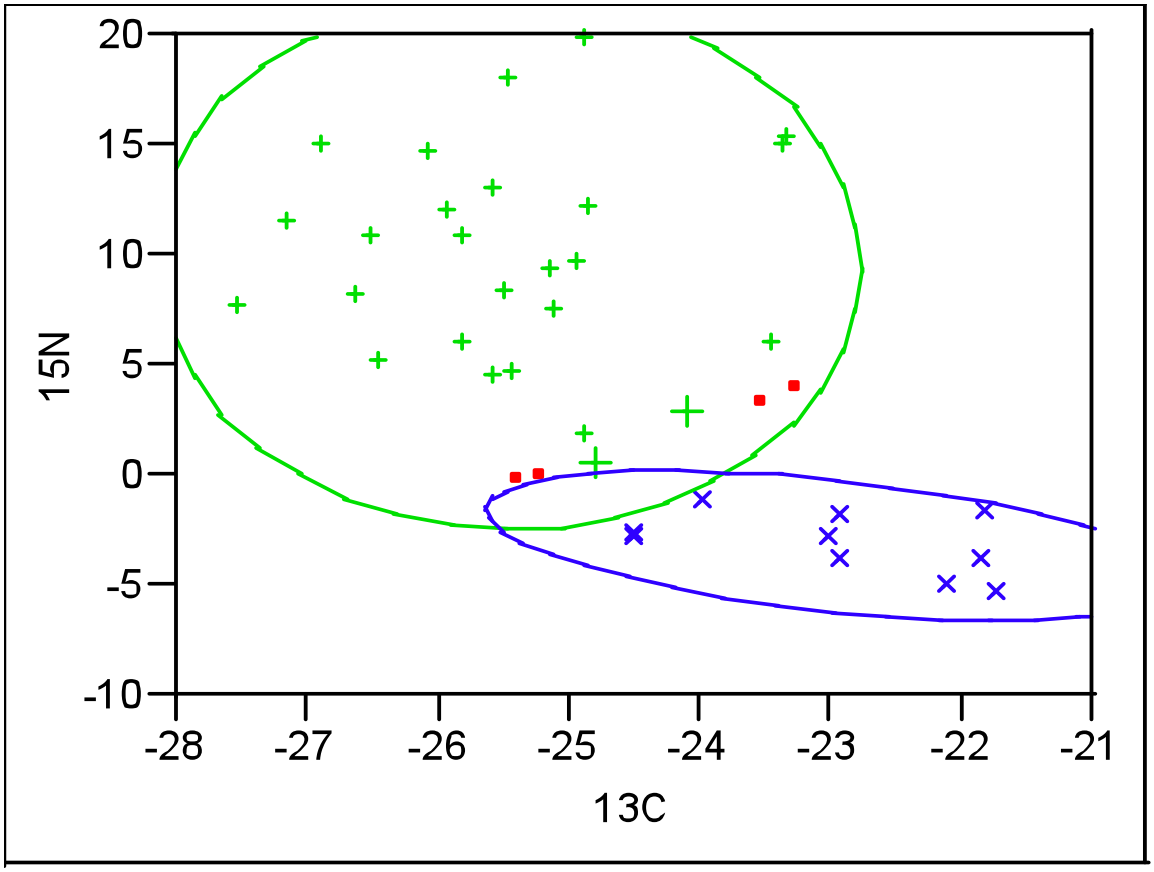


Figure 2.4 Scatterplot of $\delta^{15}N$ and $\delta^{13}C$ values from known ectomycorrhizal (green +) and saprotrophic (blue x) sporocarps.
A 95% confidence interval elliptical circle surrounds each set of values. Small red squares represent isotopic signatures of the four *Alloclavaria purpurea* sporocarps. The two large green crosses are the samples wrongly classified as saprotrophic by Discriminant Analysis.

2.4 Discussion

2.4.1 Effects of CWD retention on the ectomycorrhizal fungal community

Our study detected a slight difference in the ECM fungal community found on ectomycorrhizae of regenerating spruce between plots with and without CWD retained at harvest. In particular, *Amphinema byssoides* mycorrhizae were more abundant in removal plots and *Alloclavaria purpurea* mycorrhizae were an indicator of removal plots. This shift in species composition occurred in less than fifteen years, while the wood was still hard and intact, and even though roots in close proximity to the logs were not specifically targeted. Shifts in the ECM fungal community based on the retention of CWD have not previously been detected (Olsson et al., 2011), even though some ECM fungi seem to preferentially form mycorrhizae in decayed wood (Goodman and Trofymow, 1998) or fine woody debris (Buée et al., 2007), and the loss of wood-dependent fungal species has been documented in the absence of retained down wood (Berg et al., 1994). The volume of CWD in past studies was very low, with small differences between treatments (e.g. retention volumes of ≤ 60.4 $m^3 ha^{-1}$, and removal volumes between 0 and 4 $m^3 ha^{-1}$) (Olsson et al., 2011). Our study may have established some threshold volume necessary to detect a shift in ECM root tip community composition between CWD retention and CWD removal plots, at least for subalpine conifer forests. Retention volumes ranged from 420-450 $m^3 ha^{-1}$, and removal volumes were approximately 60 $m^3 ha^{-1}$ for this study. CWD covers 14% of the ground surface, is dominated by pieces larger than 12 cm in moderate

stages of decay (i.e. they still maintain their original shape), and is estimated to remain on the ground for an average of 320 to 350 years in mature forests at the Sicamous Creek study area (Feller, 1997). In Sweden, where decades of leaving a bare forest floor post-logging has led to the loss of many wood-dependent fungal, lichen, bryophyte, and invertebrate species, 30% of natural CWD levels has been determined as a threshold below which species will be lost (Berg et al., 1994). In British Columbia, operational guidelines, such as retention of 50% of natural CWD levels, have been suggested since the implementation of the Forest and Range Practices Act in 2004 (Bunnell and Houde, 2010), but these are not legally binding and are rarely met (BC Ministry of Forests and Range Chief Forester, 2010). This study suggests that the difference between as little as 10% of natural downed wood remaining on removal plots, versus 50 % to 100 % of natural levels on retention plots, can influence the ECM fungal community.

Ectomycorrhizae are formed by different fungi on seedlings growing in clearcuts versus forests (Dickie et al., 2009; Jones et al., 2003). Some of this shift appears due to changes in soil chemistry, such as increases in mineralizeable N and higher soil C (Dickie et al., 2009). Decaying CWD does contribute to higher soil C and thicker organic layers in the forest floor and upper soil horizons; however, its influence on soil chemistry is detectable only at advanced stages of decay (Kayahara et al., 1996, Laiho and Prescott, 1999; Spears et al., 2003; Spears and Lajtha, 2004). Hence, it was somewhat surprising that we detected an influence of CWD on the ECM root tip community in the medium term, while the

CWD was still hard and intact. From the perspective of ECM fungi, however, the main influence of *intact* CWD is to moderate temperature and moisture fluctuations by shading nearby soil (reviewed by Bunnell and Houde, 2010), and to provide surfaces for hymenium formation for resupinate species (Olsson et al., 2011; Tedersoo et al., 2003, 2009). Soil temperatures in clearcuts at Sicamous Creek can vary by more than 15 °C on a daily basis (J Walker, unpublished data), and any amelioration of these extremes by large intact CWD would be expected to influence colonization of fine roots since ECM fungi differ in their ability to grow and form ectomycorrhizae at high temperatures (Cline et al., 1987; Parke et al., 1983a) and to persist or regenerate at low soil moisture (Izzo et al., 2005; Parke et al., 1983b).

Atheliod fungi, including *Amphinema, Tylospora*, and *Piloderma*, are generally expected to be strongly associated with wood (Tedersoo et al., 2003), although Allmér et al. (2009) found *Amphinema* hyphae more frequently in spruce-needle bait bags than in wood. We found an elevated abundance of *Amphinema byssoides* ectomycorrhizae in removal plots, which is surprising, especially given that this species fruits on downed wood (Tedersoo et al., 2003), is more abundant in organic litter layers than upper mineral soil horizons (Baier et al., 2006), and does not tolerate elevated temperatures (Kipfer et al., 2010). *A. byssoides* has been shown to outcompete co-occurring inoculant ECM fungi on five-year-old seedlings in clearcuts (Gagné et al., 2006), and to infiltrate the forest ECM community when transplanted from the disturbed soils that it

dominates (Kranabetter, 2004), but we do not know of any studies that have shown it to be particularly adapted to sites with few organic inputs or woody cover. *A. byssoides* may be adapted to plots with fewer legacies of mature forests, since its ability to disperse to and colonize seedlings in nurseries (Rudawska et al., 2006) and disturbed substrates categorizes this fungus as a ruderal species (Hagerman and Durall, 2004).

The species identified as an indicator for removal plots, *Alloclavaria purpurea* has been assumed to be a saprotroph. If this were true, we would expect it to be more strongly aligned with plots containing higher volumes of CWD. Our cloning of PCR amplicons from ectomycorrhizae at Sicamous Creek indicates that *A. purpurea* is an ECM fungus. Additionally, we found that it was much more abundant (relative to other ECM taxa) on root tips than as hyphae, which suggests that it is an especially successful colonizer of fine roots. The ECM fungus *Coltricia perennis* was recently included in the Hymenochaetoid clade along with *A. purpurea* (Dentinger and McLaughlin, 2006). The nutritional mode of *Calostoma cinnabarinum* was recently confirmed to be ECM based on its ^{13}C signature even though this member of the Boletales was previously thought to be saprotrophic (Wilson et al., 2007). The $\delta^{13}C$ and $\delta^{15}N$ of *A. purpurea* sporocarps from our study was within the range of other ECM fungi from the site, but was peripheral enough in that range that a discriminant analysis did not classify it as such. Further experimentation is required in order to confirm that *A. purpurea* is ectomycorrhizal. This would require an unambiguous morphotype description,

and a successful test of Kochs postulate. Nevertheless, we conclude that *A. purpurea* is forming ectomycorrhizae at Sicamous Creek based on our molecular and isotopic evidence.

2.4.2 Comparison of ECM fungi on ectomycorrhizae and in mesh bags

OTU-based grouping by MOTHUR (molecular similarity) and phylogenetic-based naming in MEGAN (taxonomic similarity) were used together as tools to identify ECM fungal species, genera, and family groups in all experimental plots for both ECM root tips and mesh bag hyphae. Sequence processing, filtering, and grouping were based on a combination of steps gleaned from other studies exploring this technique (Amend et al., 2010; Buée et al., 2009; Jumpponen and Jones, 2009; Tedersoo et al., 2010b). Although we conservatively clustered the root tip and mesh bag sequences into OTUs based on 95% similarities (Jumpponen and Jones, 2009), it resulted in a higher number of fungal groups than other studies of fungi in forest soils (Buée et al., 2009; Lim et al., 2010; Tedersoo et al., 2010b). Sanger-based sequencing of fungal DNA from ECM root tips and pyrosequencing of fungal hyphae from sand-filled mesh bags detected similar numbers and identities of ECM fungal taxa in total at this site in spite of the vast difference in numbers of sequences generated by the two approaches. We had expected to detect far more ECM taxa, especially rare species, from the > 120,000 reads (sequences) from hyphae in 180 mesh bags than from 248 extractions of ectomycorrhizae from 60 root samples. Tedersoo et al. (2010b) found that careful molecular analysis, especially the critical examination of

singletons, resulted in comparable views of the ECM community even though pyrosequencing generated an enormous amount of data. In our study, twice as many ECM taxa were detected as hyphae than as root tips within each plot, reflecting differences between the two growth forms of ECM fungi in the distribution of their habitats and the extent to which species can intersperse in those habitats. The space for fungal hyphae is essentially unlimited in soil, while the opportunity for colonization on root tips is limited by the number of short roots; therefore, only a few of the ECM taxa that may very well be present as hyphae in a sample will actually occupy (and be detected on) immediately adjacent root tips. Furthermore, a single conifer short root is most commonly colonized by one ECM fungus, whereas tens of ECM fungi can co-occur in a mm^3 sample of forest soil (D.B. Brooks and M.D. Jones, unpublished data). Even though our sampling of the root tip community was extensive enough that site-level root tip ECM richness closely approximated that of the hyphae ECM richness, it gave a biased view of ECM species common to all plots, and misrepresented the true distribution of ECM taxa among plots. This is why ordinations of presence-absence data appear to show that some root tip ECM taxa are uniquely aligned with one plot, and only a few taxa are shared (they occupy the center of the ordination), whereas ordinations of the hyphal taxa reveal that many more taxa, including the drivers of community structure, are shared among plots.

The abundance, diversity, identity (Kjøller, 2006), and distribution (Genney et al., 2006) of extramatrical mycelia often differ from that of ECM root tips. Taxa that produce more extensive mycelia will be detected more frequently in hyphal samples than on colonized roots because extramatrical hyphae of ECM fungal species branch and aggregate to different degrees (Agerer, 2001). Kjøller (2006) found that Boletoid species occurred more frequently as mycelia than as root tips; the opposite was true for Russuloid and *Cortinarius* spp. Others have found that the relative abundance of ECM fungal communities was the same when assessed by root tips or mycelial abundance in mesh bags, aside from the detection of a few rare species by one method or the other (Korkama et al., 2007). In our study, the mesh bags did not appear to select for or against ECM taxa based on hyphae exploration type. *Russula* and *Lactarius* – both 'contact type' – were detected as both root tips and hyphae, as were *Cenococcum, Inocybe,* and *Tylospora*, which form prolific but short and diffuse hyphae (Agerer, 2001).

2.4.3 Evidence of succession in the ectomycorrhizal community at Sicamous Creek

ECM fungi that were present on the roots of mature trees at the time of logging, such as *Cenococcum* and *Piloderma* (Hagerman et al., 1999), are now present on the roots of 10-yr-old saplings. However, the dominant ECM taxa continued to be those that were major colonizers of the spruce seedlings in the nursery, such as *Thelephora terrestris*, *Amphinema* sp., and *Wilcoxina* sp. (Jones et al., 2002).

Thelephora terrestris was still the most abundant species on ECM root tips at the time of this study, while *A. byssoides* was the most abundant species in mesh bags. The former was the most abundant ECM fungus on seedling roots in all three years after outplanting (Jones et al., 2002). Spores of *T. terrestris* are known to rapidly colonize sterilized substrates in forest nurseries (Smith and Read, 2008), and indigenous *T. terrestris* can quickly colonize pre-inoculated roots in disturbed systems (Kranabetter and Friesen, 2002). The same high dispersal rates and ability to outcompete other EMF in nurseries and on naturally colonized conifer seedlings are observed for *A. byssoides* and *Wilcoxina* spp. (Aucina et al., 2007; Gagné et al., 2006; Jones et al., 2010; Kranabetter, 2004; Rudawska et al., 2006). *Lactarius* spp., *Piloderma* spp., and *Cenococcum geophilum* were detected by Jones et al. (2002), but only on 1 % of nursery seedling roots. These fungi were still in very low abundance on root systems of the 10-yr-old saplings, and tend to be more characteristic of mature forests. Therefore, forest taxa are not well represented, and there is little evidence that the ECM community has begun to shift back to that of the original forest after almost 15 yrs. We propose that the Sicamous Creek site is still in a post-disturbance successional stage. *Thelephora* mycorrhizae are still dominant, while some forest taxa are moderately abundant (*Tylospora*, *Inocybe*), and some are beginning to recover (*Lactarius*, *Pseudotomentella*). Other taxa are perhaps still in transition (*Russula*, *Cortinarius, Piloderma*) (Twieg et al., 2007), or remain under-detected due to the limitations of current molecular techniques (*Cenococcum*) (Kauserud et al., 2011).

Our observations of ECM succession on the same site are unique; most previous studies have used chronosequences of sites, often differing in original disturbance type. These studies have concluded that pioneer fungi are not completely replaced. Instead, an increase in the complexity of the community gradually occurs, with diversity reaching a plateau in some systems after 26 years, and changes in community composition stabilizing after 65 years (Twieg et al., 2007; Visser, 1995). These observations are related to the time of canopy closure in rapidly regenerating Douglas-fir (Twieg et al., 2007) and jack pine (Visser, 1995) stands. High elevation spruce-fir forests are long-lived and slow-growing (Hollstedt and Vyse, 1997), therefore we may not have detected a shift in the ECM community because more time may be required for the host tree community to regenerate in our system.

It is accepted that the ECM fungal community that initially establishes in clearcuts is not the same as that colonizing seedlings in forest because the fungi that first colonize seedlings in clearcuts appear to colonize primarily by resistant propagules or spores, and therefore dominate when living inoculum is reduced or absent (Jones et al., 2003). Pioneer taxa, whether endemic to the site or introduced on nursery seedlings, may persist and dominate in clearcuts because initial colonizers can retain a competitive advantage, especially when they occupy a large proportion of the root system (Kennedy et al., 2009). Furthermore, there may have been little change in the ECM community on spruce roots over the 9 years since the previous sampling because many of the

dominant fungi are nitrophiles (Kranabetter et al., 2009) and they may still be better adapted to edaphic conditions in this young stand. Evidence for this includes higher rates of accumulation of labeled N by spruce seedlings colonized by *Wilcoxina* sp. or *Amphinema byssoides* than *Cenococcum* sp. at Sicamous Creek (Jones et al., 2009). The detection of most ECM taxa as hyphae in mesh bags confirms that they are alive in the soil, and suggests that they are not limited by spore dispersal or the resilience and distribution of other resistant propagules (Peay et al., 2010).

3 Ectomycorrhizal fungus root tip community structure and enzyme activity varies among forest and clearcut plots, but not among decayed wood, downed wood, and mineral soil microsites

3.1 Synopsis

The ectomycorrhizal (ECM) fungi that colonize seedlings in forests are different from those in clearcuts (Dickie et al., 2009; Ding et al., 2011; Jones et al., 2003; Mah et al., 2001) because of limited inoculum (Dickie and Reich, 2005; Hagerman et al., 1999; Izzo et al., 2006; Peay et al., 2010; Taylor and Bruns, 1999; Tedersoo et al., 2008) changes in available hosts (Ding et al., 2011; Tedersoo et al., 2008), competition for fine roots (Kennedy et al., 2009; Koide et al., 2011), and altered abiotic properties (Dickie et al., 2009; Ding et al., 2011; Peay et al., 2010; Jones et al., 2003). Downed logs influence the environment for ectomycorrhizal (ECM) fungi (Bunnell and Houde, 2010; Elliot et al*.,* 2007; Harvey et al., 1979; Kayahara et al*.*, 1996; Laiho and Prescott, 2004; Spears et al., 2003; Spears and Lajtha, 2004; Tedersoo et al., 2003), and in a decayed state, the downed wood is penetrated by the roots of tree seedlings and their ECM symbionts (Christy et al*.*, 1982; Harmon et al., 1986). Therefore, by increasing habitat diversity, and acting as a source of inoculum, decayed and downed wood may be especially important for preserving forest ECM species in young disturbed stands (Smith et al., 2000).

The secretion of hydrolytic and oxidative enzymes by ECM fungi contributes to nutrient cycling in temperate forests (Luis et al., 2005; Molina et al., 2008; Schimel and Bennett, 2004), and the study of enzyme profiles associated with ECM fungi can provide information on the functional diversity of an ECM fungal community (Buée et al., 2007; Courty et al., 2007; Jones et al., 2009, 2010, In review; Rineau and Courty, 2011). This knowledge is valuable for forest management if the loss of ECM taxa results in decreased fitness for regenerating seedlings due to diminished ECM community physiological function.

The first objective of this chapter (Objective 3 of the thesis) was to determine whether the composition and physiological activities of ectomycorrhizae differ among microsites of decayed wood, mineral soil, or adjacent to hard downed wood in clearcuts, and their similarities to those in forest microsites. I predicted that woody debris would influence the development of ECM fungal communities in clearcuts by retaining forest species and by providing suitable habitat. I tested this by examining the composition of the ECM fungal community on the roots of spruce seedlings in microsites of decayed wood, adjacent to hard downed wood, or in mineral soil in both forest and clearcut plots. Additionally, I measured a number of abiotic properties, and the activity of depolymerases that are involved in organic matter breakdown. The second objective of this chapter (Objective 4 of the thesis) was to explore the capacity of individual ECM fungal taxa for plasticity among microsites, and to determine if there is evidence of functional complementarity among species that co-occur in the same microsite. I proposed

that if communities of ECM fungi differed among microsites because they were adapted to accessing nutrients from organic matter in those substrates, I would expect activities of depolymerases would also vary. I expected to observe physiological plasticity in dominant ECM taxa for these extracellular enzyme activities in response to changes in the different microsites. I also expected to observe functional complementarity among ECM fungi in the same microsite, in addition to some functional overlap.

Hypothesis 3: Taxonomic and functional differences will be found in the ECM root tip community among soil microsites, but those from decayed wood will be most similar to those in forest plots.

Hypothesis 4: Individual ECM fungal species will show altered patterns of enzyme activity in different microsites, and co-occurring ECM fungal species will show different and complementary patterns of enzyme activity in the same microsite.

3.2 Methods

3.2.1 Field site description and experimental design

The experimental area investigated in this chapter includes the three replicate 10 ha clearcut blocks harvested at Sicamous Creek in the winter of 1994/95. Inside each of these are two 1 ha treatment plots, one where coarse woody debris was retained and one where coarse woody debris was removed. The treatment plots

were established in 1996. In 2007, I established a 1 ha treatment plot in the undisturbed forest, adjacent to the southern margin of each cutblock, and 30 m inside the forest edge to minimize edge effects and to avoid blowdown.

I grew hybrid *P. engelmannii* x *Picea glauca* (Moench) Voss (native interior hybrid spruce) in a greenhouse from seed collected from the same biogeoclimatic sub-zone, and the same elevation as the Sicamous Creek site (seedlot number 26212; B.C. Ministry of Forests Seed Center, ID DWD 20070064A; collection elevation mean 1675 m), beginning in early May 2007. I planted two surface-sterilized seeds (H_2O_2 30 % for 15 min, 5 % for 5 h) into 700 150 ml bleach-sterilized Ray Leach Cone-tainers (Stuewe & Sons, Tangent, Oregon) containing autoclaved 1:1 peat:vermiculite. I covered the seeds with approximately 15 ml sterile horticultural sand. At 6 weeks, I hardened the seedlings off outdoors, and at 7 weeks, chose three seedlings at random to check root development and to confirm their non-mycorrhizal status. I sampled an additional nine seedlings throughout planting to check for colonization by looking for a fungal mantle under the dissecting microscope. None of the sample seedlings were mycorrhizal.

I planted eight-week old spruce seedlings in three different types of soil microsites at 25 randomly selected locations in every 1 ha plot throughout July 2007: control sites, downed wood sites or decayed wood. Control microsites comprised primarily mineral soil, and were located at least 50 cm from any visible

downed wood. Downed wood microsites were mineral soil within 5 cm of a piece of downed wood at least 10 cm in diameter and of the Vegetation Resource Inventory (VRI) decay class 1-3 (Government of BC VRI 2004). Decay class 1-3 ranges from hard, intact logs with bark and twigs attached, to sagging, partly decayed logs with roots invading the sapwood. The decayed wood microsites were of VRI decay class 4, 5, or beyond. These decay classes include sunken logs that are no longer round and which have roots invading the heartwood, as well as small, soft portions of wood on the ground. I planted seedlings directly into the decayed wood. At each planting location, the three microsites were within 2 m of each other, and one seedling was planted in each of these microsites. The downed wood microsites in removal plots were located immediately adjacent to major above-ground horizontal roots associated with stumps because these plots contained no logs. I covered each seedling with a 10 cm tall wire cage to reduce herbivory from rodents. All six clearcut plots experienced 30-50% seedling mortality due to very hot and dry conditions; seedling survivorship was significantly different between forest and clearcuts plots (it was higher at forest plots), but it was not significantly different among microsites within plots. I replaced over two hundred seedlings in two subsequent visits to the site in the first and second week of September using a second batch of non-mycorrhizal seedlings. I grew these in the same conditions and from the same seedlot as the original set of seedlings. I replaced all dead seedlings; surviving seedlings in other microsites at the same location were not replanted.

3.2.2 Seedling harvesting and root tip sampling procedures

I harvested seedlings one year later, between mid-August and the end of September 2008. I cut a 10 cm wide x 10 cm wide x 20 cm deep block of soil/decayed wood around each seedling with a pruning saw to ensure that entire root system was harvested. The block extended beyond the short lateral roots of these seedlings. I removed the entire block using bare hands to ensure that no resistance was felt at the deepest part of the cut that could signal long central roots breaking off. I put the seedling-plus-soil sample into a large plastic bag, and kept the bags sealed and on ice until they could be stored at 4 ˚C in the lab.

I harvested seedlings for physiological analysis (enzyme assays) weekly from August 11th through September 6th 2008. Each week, I collected one seedling per microsite from the same location in each treatment plot at all three blocks (1 seedling x 3 microsites x 3 plot treatments x 3 blocks = 27 seedlings). Enzyme analyses took place within 3 d of sample collection. I gently washed seedlings from the surrounding soil under tap water, and examined the entire root system under a dissecting microscope for number and condition of colonized root tips (mycorrhizae). I froze the soil remaining in each sample bag for future chemical analysis. For each seedling, I removed all mycorrhizae with forceps, and grouped them according to their morphology; I selected up to 14 tips from each seedling for immediate enzyme analysis. I filled the 96-well assay microplate in order to best represent the actual proportion of different morphological groups found on

each seedling. For example, I first filled wells with morphotypes that had only one representative, then with those that had two or more. Typically, there were only 3-4 groups, one of which was far more abundant than the others. In general, only a few wells were occupied by the rare morphotypes (e.g. morphotype 1 in the first three, morphotype 2 in the next four), and the rest of the wells (i.e. approximately half) were filled with the dominant morphotype. This allowed the most abundant group to be adequately represented, while ensuring that rare taxa were not overlooked. If fewer than 14 tips were present on a seedling, all tips were assayed. Once assays were complete (see below for details) I froze all root tips at -80 °C for DNA analysis to confirm identities of the mycobionts.

I collected five seedlings per microsite per plot for community analysis (morphotyping and subsequent molecular identification) from September 22-24, 2008. I harvested and stored each seedling as outlined above, except that I was not always able to collect all three seedlings from the same original planting location (i.e. because of additional seedling mortality, some locations had only one seedling in one microsite type still alive). There was sufficient replication however, because seedlings were originally planted in far more locations (25) than required for this study (10); representative seedlings from the missing microsite type required could always be in another planting location in the same plot. I completed morphotyping of all live tips per seedling within 6 months, based on Agerer's (1987-2002) descriptions and the instructions of Goodman et al. (1996). I distinguished morphotypes by the type of branching, colour, texture,

abundance of hyphae, presence of rhizomorphs, and other microscopic features of the mantle (e.g. mantle pattern) and emanating hyphae (e.g. types of connections) under both dissecting (50X and 100X) and compound (1000X) microscopes. I froze two representatives of each morphotype per seedling at -80 °C for DNA extraction and molecular identification; I retained soil immediately adjacent to each seedling for chemical analysis.

3.2.3 Soil abiotic properties

I recorded volumetric soil moisture and soil temperature for all three microsites at all retention and forest plots. In the center of each plot, I installed one Decagon 'Em5b' datalogger with three 'ECH2O' soil moisture sensors attached (both Decagon Devices, Inc. Pullman Wa), and buried three Onset 'Stowaway Tidbit' temperature loggers (Onset Computer Corporation, Pocasset, MA). I buried the three pairs of sensors 5 cm deep immediately adjacent to the seedling planted at each of three microsites at one planting location.

I ground air-dried subsamples (135 x @10 g) of the bulk soils surrounding each enzyme assay seedling with a mortar and pestle, and these were individually tested for mineralizeable N (ammonium, 1 M KCl anaerobic incubation), available nitrate-N and ammonium-N (2 M KCl extraction), available phosphate-P (Bray P-1), and total C and N (combustion elemental analysis). These tests were performed at the Ministry of Forest and Range, Analytical Chemistry Services. I

also analysed air-dried and ground subsamples of the enzyme assay seedling soils for pH (H_20) in-house.

3.2.4 Enzyme assays

I conducted enzyme assays on individual root tips in a 96-well microplate as outlined in the methods developed by Pritsch et al. (2004) and Courty et al. (2005). I tested eight extracellular enzymes: acid phosphatase (EC 3.1.3.2), β-glucosidase (EC 3.2.1.3), β-glucuronidase (EC 3.2.1.31), leucine aminopeptidase (EC 3.4.11.1), β-xylosidase (EC 3.2.1.37), cellobiohydrolase (EC 3.2.1.91), *N*-acetylglucosaminidase (EC 3.2.1.14) and laccase (EC 1.10.3.2) (Table 3.1). I placed each of 14 tips per seedling into individual micro-sieves constructed from 200 µl PCR tubes and held together such that they could be immersed and withdrawn from substrates in the microplates (see Pritsch et al., 2004), then rinsed and reinserted into new substrate. By performing the steps this way, each tip was assayed sequentially for each enzyme. The tips from each seedling occupied two columns of the plate, and the final row was left as a blank. I made up working solutions of the fluorimetric and colorimetric (laccase) substrates weekly, and stored them in the dark until I used them for assays. I kept tips in their micro-sieves in a plate of rinse buffer for at least 5 min, then immersed them in substrate in an incubation plate at 100 rpm and 21 ºC in the dark. I removed the sieves and immersed them in a plate of stop buffer (pH 10.5) after a pre-determined length of time. After a further 5 min rinsing, I placed the sieves in the next substrate. Concurrently, I read aliquots of the incubation solution on a

FLUOstar Galaxy fluorescent microplate reader (BMG Lab Technologies, Ortenberg, Germany). I scanned root tips with Scanmaker 8700 (Microtek Lab Inc., Santa Fe Springs, CA) once assays were complete to determine projected surface area using WinRHIZO (Regent Instruments, CANADA), then froze them at -80 ºC for future molecular identification.

Table 3.1 Enzyme names used in this thesis, their assay substrate, and the component on which they act in soils.

Enzyme name	Assay substrate	Acts on
xylosidase	4-MU[1] ß-D-xylopyranoside	hemicellulose
glucuronidase	4-MU-ß-D D-glucuronide hydrate	hemicellulose
cellobiohydrolase	4-MU ß-D-cellobioside	cellulose
glucosidase	4-MU ß-D-glucopyranoside	cellulose
chitinase	4-MU N-acetyl-ß-glucosaminide	chitin
aminopeptidase	L-leucine 7-AMC[2]	proteins and peptides
phosphatase	4-MU phosphate free acid	organically bound P
laccase	ABTS[3]	phenolics (e.g. lignin)

[1]4-methylumbelliferone
[2]7-aminomethylcoumarin
[3]Diammonium 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate)

3.2.5 Molecular identification of fungi from all ectomycorrhizae

I extracted fungal DNA from frozen root tips by following the protocols of the Sigma Extract-N-Amp Plant PCR Kit (Sigma Aldrich, St. Louis MO, U.S.A.), and amplified the Internal Transcribed Spacer (ITS) region of nuclear rDNA amplified with GoTaq (Promega Corporation, Madison WI, U.S.A.) using the forward primer ITS1F (5'- CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns, 1993) and the reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). This primer pair amplifies both ascomycete and basidiomycete DNA. The 50 μl

GoTaq reaction mixture included 2.5 μl of each 10 μM primer, 25 μl of GoTaq master mix 2X, 18.5 μl of sterile water, and 1.5 μl of template DNA. Thermal cycler conditions were: a 3 min initial denaturation at 94 °C, followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, and a final 10 min extension at 72 °C. I visualized amplicons on a 1 % agarose gel, cleaned single bands with Agencourt AMPure XP magnetic beads according to the 96-well plate procedure (Beckman Coulter, Beverly MA, U.S.A.), and quantified them with a NanoDrop micro-volume spectrophotometer (Thermo Scientific, Wilmington DE, U.S.A.) prior to in-house Sanger sequencing. Amplicons were sequenced with forward primer ITS1F and reverse primer ITS4 using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems Inc, Foster City, CA, U.S.A.).

I aligned and corrected sequence contigs of fungal DNA from ectomycorrhizae using Sequencher 4.2 (Gene Codes Corp, Ann Arbor, MI, U.S.A.), and removed primers with MOTHUR v. 1.16.0 (Schloss et al., 2009). I isolated the entire ITS region using the Fungal ITS Extractor (Nilsson et al., 2010) and compared it against the GenBank nucleotide database (BLASTn, Altschul et al., 1997) via the ITS Pipeline (Nilsson et al., 2009), which groups sequences taxonomically. Specifically, the ITS Pipeline matches fungal ITS sequences to the GenBank database, and then groups those that share 50 % of their top fifteen closest BLAST database hits, based on their taxonomic names. I also aligned the ITS sequences with MAFFT v. 5 (Katoh et al., 2002), then assembled them into distance matrixes and clustered them in MOTHUR (countends =F, cutoff=0.10).

Specifically, fungal ITS sequences are compared to each other based solely on their DNA alignment because those that match more closely (i.e. their sequences line up) are, in theory, more closely related. The appropriate clustering cutoff can only be determined, however, by plotting a curve of the accumulation of these groups (or operational taxonomic units – OTUs) across a range of cutoffs for a given number of reads. Since I expected the best curve to occur with no less than 90 % similarity, I minimized the computing requirements by setting a 0.10 cutoff. For example, after aligning the sequences with the MAFFT software, I imported them into MOTHUR (which assembles them into distance matrixes), and subsequently clustered (grouped) them based on 90 % through 99 % base pair similarity. At 91 % molecular similarity, the resulting OTU accumulation curve was flat, suggesting that the clustered groups were too broad and inclusive, and that the asymptotic curve falsely showed that all taxa had been discovered (which is highly unlikely for ECM fungi). Conversely, the curve rose exponentially at 99 % similarity, which suggested that the cluster was too exclusive, and therefore greatly overestimated taxon diversity. After I plotted the range of curves for data from this site, I determined that 95 % molecular sequence similarity, although conservative, gave the best estimate of the number of fungal taxonomic groups. It is more common to use 97 % (Buée et al., 2009; Tedersoo et al., 2010b), but 95 % is also used (Jumpponen and Jones, 2009), and the correct choice is often ambiguous (Amend et al., 2010). As with much of this technique, limitations abound, and clear guidelines are just emerging (Avis et al., 2010; Dickie, 2010; Nilsson et al., 2006, 2009, 2011). I subsequently assigned these

root tip sequence groups a species name if the sequence had 97 % similarity over at least 450 bp to a vouchered database match, a genus name if there was 94-96 % similarity, a family name for a 91-93 % match, and an order, class or division name if < 90 % similarity.

3.2.5.1 Additional identification of ECM fungi on root tips used in enzyme assays

To minimize the amount of amplicon sequencing required, and to confirm that replicate morphotype tips assayed for enzyme activities from each seedling were indeed colonized by the same ECM fungus, I used restriction fragment length polymorphism (RFLP) enzymatic digests. After the enzyme assay, I extracted DNA from replicate tips from each seedling, amplified it as outlined above, and digested the amplicons from three to seven of the tips with the restriction enzymes *Alu*1, *Mbo*1 and *Hinf*1 (Invitrogen Corp., Carlsbad CA). A single reaction mixture included 1.0 μl H_2O, 1.5 μl React buffer, 0.2 μl BSA, and 0.3 μl enzyme, plus 7 μl template DNA; this was incubated for three h at 37 °C. I visualized the unique fragment patterns on 1.5 % agarose gels and matched them to tips on the same gel that were identified by DNA sequencing. Each unique pattern on every gel had a representative that was conclusively named. I found three replicates to be sufficient to determine that replicate tips were identical, and, as *Alu*1 frequently did not cut, or was not diagnostic (i.e. it generated the same length fragment for al tips), it was no longer used. I then matched up the named tips with the enzyme activity profile of every seedling. I

calculated activity profiles for each taxon using data from all tips that I could identify.

3.2.6 Statistical analyses

The experimental design was a balanced hierarchical model with three factors: block (A, B, C), plot treatment (forest, downed wood retention, downed wood removal), and microsite (control, downed wood, decayed wood). Block was designated as a random factor; plot treatment and microsite were fixed factors, and microsite was nested in plot. Examples of all of these analyses are available in the appendix, and are referred to in their relevant sections in the results.

3.2.6.1 Analysis of soil properties and enzyme activity

Root tip enzyme activities per seedling and soil property tests were fully balanced, with 5 seedlings (or soil samples) x 3 microsites x 3 plot treatments x 3 blocks = 135 samples. Enzyme activities are expressed per root tip, and averaged per seedling or per morphotype per seedling. Soil properties are also expressed per seedling. I assessed assumptions of a normal distribution by plotting histograms, in addition to a Shapiro-Wilk's W test, and confirmed homogeneity of variance using Cochran and Bartlett tests. I log transformed soil enzyme activities and chemical data (with the exception of pH) in order to improve normality and minimize variance. I tested these data using a mixed-effect hierarchical multivariate (Wilk's Lambda) and univariate (unrestricted over-

parameterized) ANOVA (Statistica v. 6.1; StatSoft Inc., 2003). I used post-hoc Bonferroni tests to compare differences between all pairs of means when $p<0.1$, and applied a Bonferroni correction to the interpretation of univariate results.

I attempted to test whether enzyme activity profiles differed among a few abundant ECM taxa by using the mean data per microsite per plot treatment (i.e. block was eliminated) in order to achieve sufficient replication and to directly compare taxa that had an uneven number of seedling replicates per microsite (i.e. 3-5). This resulted in n=3 plot samples per taxon (i.e. one from each microsite) or n=3 microsite samples per taxon (i.e. one from each plot) for use in one-way ANOVAs. This allowed me to make limited comparisons among taxa at the plot scale, but none at the microsite scale.

3.2.6.2 ECM fungal community analysis

For community analysis, each plot contained at least n=3 observations (i.e. seedlings) from each microsite once I identified all mycorrhizae (i.e. of the five seedlings collected from every microsite type in every plot, I was able to identify the ECM community on at least three of them, and this information was then available for subsequent community analysis). Analyses of community composition are based on the relative abundance of a fungal taxon per seedling. I calculated relative abundance by dividing the number of mycorrhizae from each taxon per seedling by the total number of mycorrhizae counted on that seedling.

I tested these data using a mixed-effect hierarchical multivariate (Wilk's Lambda) ANOVA in order to gauge the response of the entire ECM community, and with univariate (unrestricted over-parameterized) ANOVA in order to independently test the response of individual ECM fungal taxa (Statistica v. 6.1; StatSoft Inc., 2003). I used post-hoc Bonferroni tests to compare differences between all pairs of means when $p<0.1$. Where n=1 of each microsite type per plot (e.g. rarified species richness per plot per block), or when variables were tested within one plot type (e.g. community composition among microsites in forest plots only), the experimental design was no longer hierarchical, and I used a main effects (or one-way) ANOVA.

I calculated rarefied observed and estimated root tip taxon richness, diversity and evenness per samples in EstimateS (V 8.2) (Colwell, 2009). I applied sample-based rarefaction with replacement in order to correct richness estimates for the unequal number of tips per sample. I selected rarefaction with replacement rather than without, because the variance does not approach zero; however, a negative attribute of rarefaction with replacement is that the output does not reflect the actual number of taxa (Colwell, 2009). I used Coleman rarefaction to illustrate observed taxon richness, and selected Chao 1 (classic) and Jackknife 1 as the best estimators of taxon richness because they incorporate the rare species known to characterize our dataset (Chao, 2005). I used Shannon and Simpson indexes to calculate taxon evenness. I used Indicator Species Analysis, as defined by Dufrêne and Legendre (1997), in PC-ORD (McCune and Mefford,

1999), to explore the importance of individual fungal taxa to decayed wood, downed wood, and control microsite communities.

I visually explored the relative relationship among root tip communities in different plot treatments and microsites, using both taxon presence-absence and relative abundance, with Nonmetric Multidimensional Scaling (NMS) in PCORD v. 5.0 (McCune and Mefford, 1999). NMS does not assume linearity among variables (Kenkel 2006; McCune and Grace, 2002) and, because NMS is iterative and is based on ranked distances, the bias introduced by a large number of zero values is eliminated (McCune and Grace, 2002). I constructed biplots in order to highlight the environmental variables strongly structuring the ordinations. Biplots are a way of showing sample units (communities per microsite per plot in this case) and variables (e.g. soil chemical properties) in one image, although interpretation is limited because variables are not weighted (Kenkel, 2006). Ordinations using PCA document covariance among linear variables such as those generated by the transformation of environmental data (Kenkel, 2006), however, ordination of heterogeneous community data that is characterized by non-linear species response to underlying variables is not amenable to this method (Kenkel, 2006; McCune and Grace, 2002) without data transformation (Borcard et al., 2011; Kenkel, 2006).

3.3 Results

3.3.1 Abiotic properties of microsites and plots

There were significant differences in chemical properties among microsites and among plot treatments for soils collected with seedlings used for enzyme analysis (Table 3.2). Overall significant differences in soil chemistry among microsites were detected for Total C, Total N, available phosphate-P, available ammonium-N, available nitrate-N, mineralizeable N, and pH when microsites were tested as part of the hierarchical model (Table B.1). In general, C, N, and other mineral nutrients were lower in control microsites than beside downed wood or in decayed wood, but variation was high, and differences were significant in only some plot treatments. Soil pH was highest in control microsites, and differences between control and wood microsites were most consistently observed in removal plots (Table 3.2). Total C, Total N, available phosphate-P, available nitrate-N, and mineralizeable-N tended to be highest in forest plots; pH was lowest in forest plots and intermediate values were often found in removal plots (Table 3.2).

Table 3.2 Abiotic properties of soils surrounding seedlings harvested for enzyme assays. Values represent raw means (SD) for pH, Total C and N (%), or back-transformed data from the natural log for mineral nutrients. All tests were run on log-transformed data (except for pH), but extreme variation in the raw data obscured meaningful interpretation. Letters following numbers in each column show significant differences at $p \leq 0.10$ according to a post-hoc Bonferroni test.

			%		mg kg^{-1}			
		pH	Total C	Total N	Avail P	Avail NH_4-N	Avail NO_3-N	Min NH_4-N
Forest[1]	Control	4.7 (0.5)	17.7 (13.4)	0.72 (0.5)	94.6	122.5	28.0	257.8 a
	Downed	4.8 (0.3)	29.9 (18.6)	1.1 (0.6)	160. 9	114.7	45.0	298.7 ab
	Decayed	4.4 (0.4)	49.6 (12.4)	0.91 (0.4)	82.1	63.6	37.8	385.4 b
Retention[1]	Control	5.4 (0.5) a	10.5 (7.8)	0.50 (0.2)	8.9	88.9 ab	11.3	202.3 a
	Downed	5.2 (0.5) a	18.1 (14.0)	0.77 (0.5)	18.9	275.0 a	19.7	236.0 a
	Decayed	4.6 (0.4) b	40.2 (21.3)	0.52 (0.2)	23.2	29.0 b	17.1	334.0 b
Removal[1]	Control	5.3 (0.6) a	9.4 (5.3)	0.45 (0.2) a	14.5 a	115.8 ab	12.4 a	205.4 a
	Downed	4.7 (0.3) b	20.3 (15.7)	0.85 (0.6) b	61.6 b	406.0 a	27.0 ab	258.8 a
	Decayed	4.6 (0.4) b	44.6 (14.9)	0.82 (0.3) ab	76.9 b	60.4 b	36.2 b	366.1 b
p (microsite)		<0.0001	0.01	0.0005	0.0002	0.004	0.007	<0.0001
Forest[1]		4.6 (0.4) A	32.4 (19.8) A	0.91 (0.5) A	107.7 A	96.3	36.2 A	309.6 A
Retention[1]		5.1 (0.6) B	23.4 (20.0) B	0.59 (0.4) B	41.0 B	106.7	15.6 B	253.6 B
Removal[1]		4.8 (0.6) AB	24.8 (19.5) AB	0.71 (0.4) AB	15.8 C	178.0	23.0 B	269.0 AB
p (plot)		0.0001	0.001	0.006	<0.0001	0.4	<0.0001	0.003
Overall mean[2]	Control	5.1 (0.5)	12.5 (8.9)	0.56 (0.37)	39.3	109.1	17.2	221.8
	Downed	4.9 (0.4)	22.8 (16.1)	0.91 (0.57)	80.5	265.2	30.6	264.5
	Decayed	4.5 (0.4)	44.8 (16.2)	0.75 (0.30)	60.7	51.0	30.4	361.8

[1]N=5 soil samples per microsite per plot, and p-values are based on a hierarchical ANOVA with microsite nested in plot.
[2]Means are provided to more easily interpret general patterns among microsites, and have no statistical significance.

I measured two aspects of soil microclimate in forest and retention (not removal) plots in order to confirm personal observations that soils in the clearcut were warmer and drier than those in the forest, and to test whether conditions in woody microsites were more like those of the forest. Soil temperature was always lowest in downed wood microsites in retention plots, but despite monthly trends, maximum daily soil temperature averaged over all days per month did not differ significantly among microsites in either plot type ($p>0.19$) (Figure 3.1 a and b). Maximum daily soil temperature was significantly higher in retention plots than it was in forest plots in July ($p=0.002$), August ($p=0.001$), and September ($p=0.002$) 2007, and in July ($p=0.001$), August ($p=0.0006$) and September ($p=0.002$), 2008 (Figure 3.1c). Minimum daily soil moisture averaged over all days per month did not differ significantly among microsites ($p>0.47$), and while soil moisture was always higher in forest plots than in retention plots, this was only weakly significant between plots in August 2007 ($p=0.09$) and July 2008 ($p=0.08$) (Figure 3.2a-c).

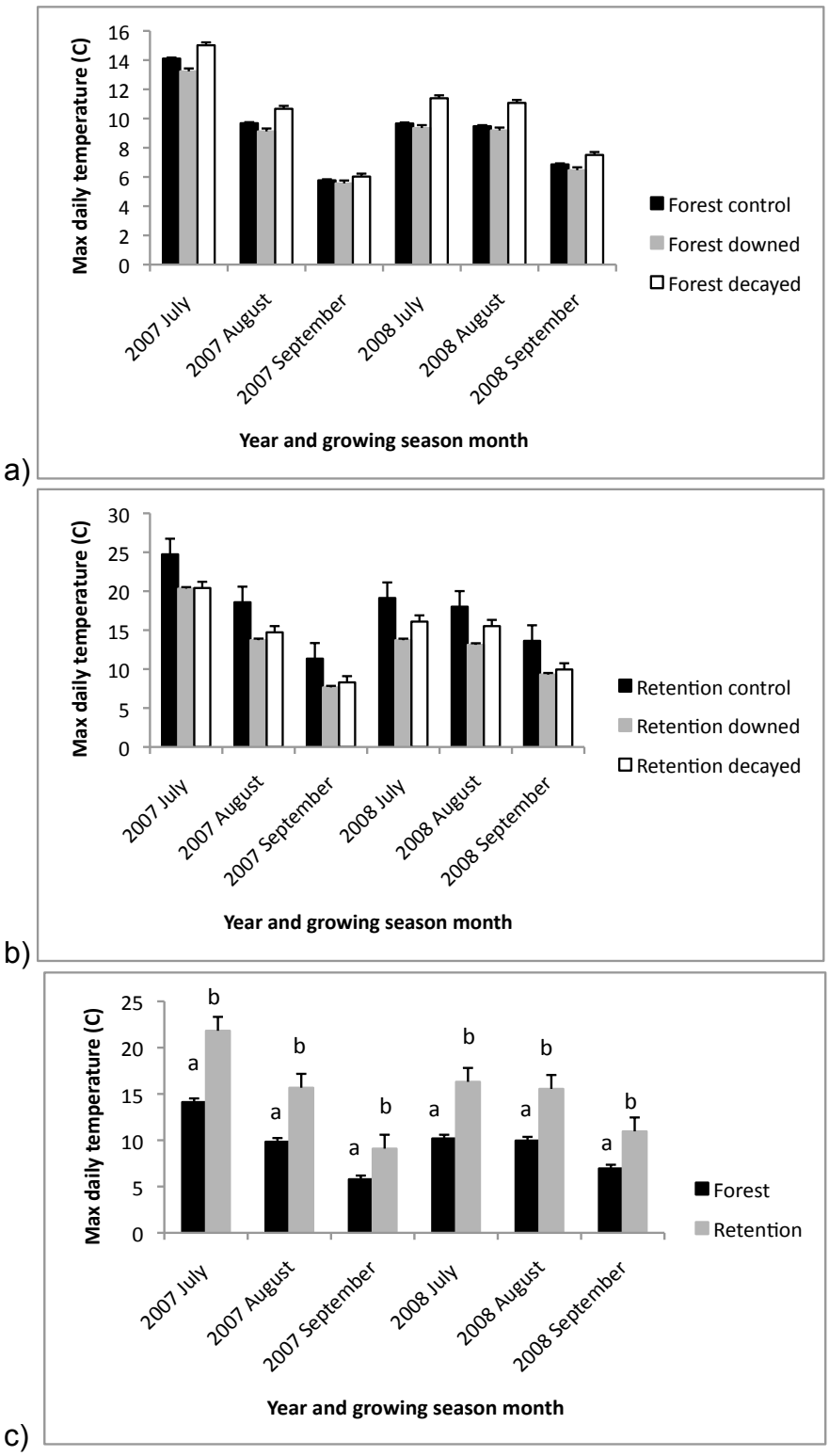


Figure 3.1 Maximum daily soil temperature averaged over 30 (September) or 31 (July and August) days per month in a) microsites in forest plots, b) microsites in retention plots, and c) between forest and retention plots.
Error bars represent +SEM and letters above columns indicate significant differences between means at $p \leq 0.10$ according to the results of hierarchical univariate ANOVAs. N=3.

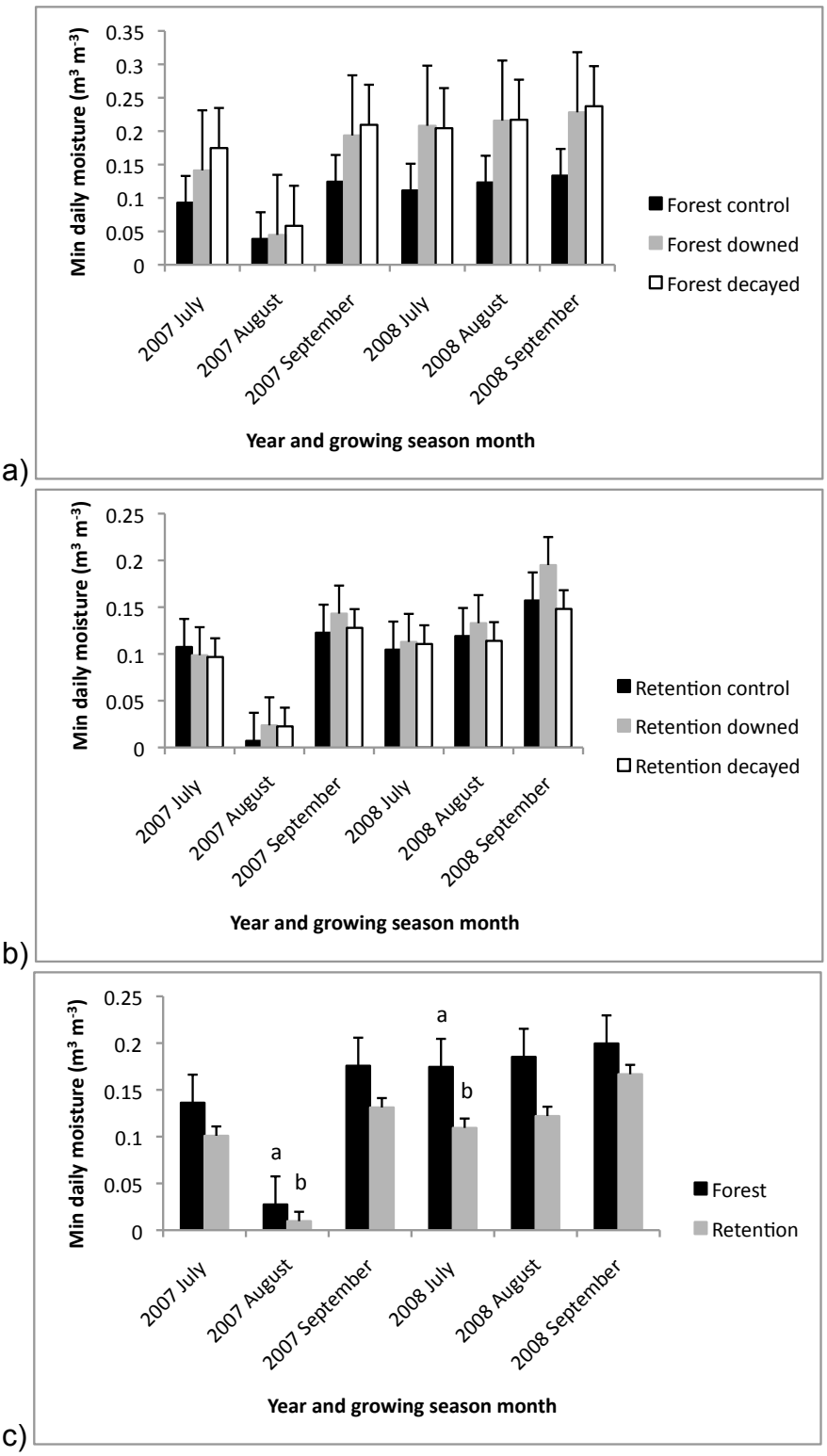


Figure 3.2 Minimum daily volumetric soil moisture averaged over 30 (September) or 31 (July and August) days per month in a) microsites in forest plots, b) microsites in retention plots, and c) between forest and retention plots for each growing season month over two years.
Error bars represent +SEM and letters above columns indicate significant differences between means at $p \leq 0.10$ according to the results of a hierarchical univariate ANOVAs. N=3.

3.3.2 Enzyme activities of the ectomycorrhizal community in microsites and plots

I examined differences in physiological traits at the community level by testing the overall community enzyme profile (i.e. the activity of all eight enzymes among all ECM root tips) in plots and microsites with a multivariate ANOVA (Table 3.3). The ECM community enzyme profile differed among plots ($p<0.0001$), but not among microsites ($p=0.21$). Univariate tests on individual enzymes confirmed that differences in ECM root tip enzyme activity were most apparent among plots, and, when considered independently (i.e. without correcting for the presence of the additional enzyme variables), laccase activity differed weakly among microsites ($p=0.03$; Table 3.4). Specifically, activity was lower in decayed wood than in control microsites in removal plots (Table 3.4; Table B.2). Activities of the other extracellular enzymes did not vary among microsites ($p >0.25$; Table 3.4). Laccase and phosphatase activities were higher in forest plots than clearcut plots, while aminopeptidase, cellobiohydrolase, and (weakly) xylosidase activities were lower in retention plots than in forest or removal plots (Table 3.4). Glucuronidase, glucosidase, and chitinase activities did not vary among plot treatments ($p\geq0.1$).

Table 3.3 Multivariate hierarchical ANOVA of community enzyme activity profiles (i.e. the activity of eight enzymes per seedling from all mycorrhizae) among microsites and plots.

	Test	Value	F	Effect	Error	p
Intercept	Wilks	0.012	1295.8	8	117	0.00
Block	Wilks	0.807	1.65	16	234	0.057
Plot treatment	Wilks	0.393	8.72	16	234	0.000
Microsite(Plot)	Wilks	0.634	1.17	48	579.8	0.206

Table 3.4 ECM root tip enzyme activity among microsites within plots, and among plots and microsite over all blocks. Values represent raw means (SD) and letters following numbers in each column show significant differences at $p \leq 0.10$ according to a post-hoc Bonferroni test. Statistically significant differences are based on log-transformed data.

		pmol methylumbelliferone (aminomethylcoumarin) $mm^{-2}min^{-1}$							mol $mm^{-2}min^{-1}$
		Glucuronidase	Xylosidase	Cellobio-hydrolase	Glucosidase	Chitinase	Phosphatase	Amino-peptidase	Laccase (^10-6)
Forest[1]	Control	0.08 (0.06)	0.78 (0.67)	1.19 (0.55)	4.27 (1.83)	5.23 (2.59)	22.9 (10.0)	1.28 (0.86)	7.80 (7.94)
	Downed	0.08 (0.05)	0.93 (0.79)	1.25 (0.67)	4.49 (2.50)	4.59 (1.57)	21.5 (9.89)	1.17 (0.34)	9.75 (8.34)
	Decayed	0.12 (0.08)	0.93 (0.75)	1.16 (1.5)	4.66 (2.50)	4.32 (1.77)	16.6 (6.84)	0.94 (0.28)	6.75 (4.64)
Retention[1]	Control	0.04 (0.03)	0.54 (0.41)	0.82 (0.46)	4.06 (2.34)	4.25 (2.94)	14.1 (6.14)	0.94 (0.51)	1.20 (1.44)
	Downed	0.05 (0.04)	0.51 (0.23)	0.77 (0.40)	3.50 (1.54)	4.55 (3.94)	16.6 (10.4)	0.87 (0.51)	1.20 (1.26)
	Decayed	0.07 (0.07)	0.72 (0.45)	0.93 (0.58)	4.04 (2.20)	5.34 (4.73)	13.4 (9.33)	0.78 (0.58)	1.50 (1.67)
Removal[1]	Control	0.07 (0.06)	0.92 (0.42)	1.30 (0.84)	5.29 (3.44)	8.04 (14.4)	20.0 (32.2)	1.10 (1.00)	1.20 (1.04)a
	Downed	0.07 (0.08)	0.84 (0.76)	1.42 (0.14)	5.06 (3.55)	5.62 (3.67)	18.4 (13.8)	1.30 (0.75)	1.20 (1.32)a
	Decayed	0.04 (0.03)	0.71 (0.61)	1.01 (0.50)	3.88 (1.86)	6.20 (5.03)	17.0 (8.53)	1.17 (1.09)	0.75 (1.12)b
p (microsite)		0.25	0.5	0.93	0.88	0.99	0.58	0.51	0.03
Forest[1]		0.09 (0.06)	0.88 (0.72) A	1.20 (0.98) A	4.47 (2.25)	4.71 (2.02)	20.4 (9.25) A	1.13 (0.56) A	8.10 (8.04) A
Retention[1]		0.06 (0.05)	0.59 (0.38) B	0.84 (0.48) B	3.87 (2.02)	4.71 (3.88)	14.7 (8.73 B	0.86 (0.53) B	1.35 (2.12) B)
Removal[1]		0.06 (0.06)	0.83 (0.61) A	1.24 (0.86) A	4.75 (3.04)	6.62 (8.93)	18.5 (20.4) B	1.19 (0.94) A	1.95 (2.02) B
p (plot)		0.21	0.03	0.006	0.19	0.1	0.005	0.009	<0.0001
Overall[2]	Control	0.06 (0.05)	0.75 (0.47)	1.10 (0.88)	4.54 (2.34)	5.84 (2.72)	19.2 (10.3)	1.10 (0.72)	3.45 (4.24)
	Downed	0.07 (0.04)	0.76 (0.53)	1.15 (0.58)	4.35 (2.16)	4.92 (3.92)	18.9 (12.2)	1.12 (0.86)	4.05 (3.98)
	Decayed	0.08 (0.06)	0.79 (0.69)	1.03 (0.46)	4.19 (2.08)	5.28 (4.24)	15.7 (8.69)	0.96 (0.36)	3.00 (4.02)

[1]n=5 seedlings per microsite per plot, and p-values are based on a hierarchical ANOVA with microsite nested in plot.
[2]The overall mean is provided to more easily interpret patterns among microsites and has no statistical significance.

3.3.3 Fungal community identification

I collected 121 spruce seedlings with active ectomycorrhizae on their root systems. Of 3518 root tips morphotyped (mean number of active ECM root tips per seedling = 27.2; SD = 21.0), I submitted 321 for molecular analysis (one tip from each pair reserved from each morphotype from every seedling), and 86.9% of these were identified. The sequences clustered into 63 OTUs, representing 2906 (82.6%) of the root tips examined. I named OTUs with as much resolution as possible based on database matches. Prior to rarifying the taxon richness, the overall mean number of taxa per seedling was 1.83; SD = 0.91.

The fungal community on bioassay seedlings at Sicamous Creek included primarily ECM taxa; I determined 44 OTUs to be ectomycorrhizal taxa, 15 OTUs to be known or suspected of forming ECM or other mycorrhizal associations, or represented fungal groups known to contain mycorrhizal species, and only 4 OTUs to be saprotrophs (Table 3.5). I did not include the four known saprotrophs in subsequent analyses. *Thelephora terrestris*, *Amphinema byssoides*, and *Tylospora* sp. 1 (likely *T. asterophora*) made up the largest proportion of identified ECM fungi colonizing spruce roots (Table 3.5). Subdominant ECM taxa included *Tylospora* sp. 2 (likely *T. fibrillosa*), *Piloderma* spp. and a member of the Pyronemataceae (likely *Wilcoxina* spp.). I detected several more ECM taxa among root tips collected for enzyme assays but I did not enumerate these for the purpose of community analyses: *Laccaria nobilis*, *L. proxima*, *Cortinarius*

obtusus, C. *olivaceofuscus*, *Russula nauseosa*, R. aff. *sapinea*, *Dermocybe semisanguinea*, *Wilcoxina rehmii* and *Meliniomyces bicolor*.

Table 3.5 Fungal taxa detected on root tips of spruce seedlings collected for community analysis from Sicamous Creek across plot treatments and microsites. Taxa are grouped by order or trophic status, and these labels are underlined.

Final OTU name	% of identified root tips	# of root tips represented	Accession number and name of best NCBI match	# of bases (%match)
Thelephorales				
Thelephora terrestris[1]	**17.80%**	650	EU427323 *Thelephora terrestris*	582/582 (100%)
Thelephora sp.	0.70%	45	FJ532478 *Thelephora terrestris*	287/291 (98%)
Thelephoraceae	2.90%	70	EU427323 *Thelephora terrestris*	452/522 (86%)
Pseudotomentella tristis	0.70%	3	AJ889968 *Pseudotomentella tristis*	597/601 (99%)
Pseudotomentella sp.	0.40%	3	AF274768 *Pseudotomentella mucidula*	499/522 (95%)
Tomentella badia	0.40%	23	AF272917 *Tomentella badia*	523/538 (97%)
Tomentellopsis submollis	0.40%	22	AJ438983 *Tomentellopsis submollis*	599/609 (98%)
Atheliales				
Amphinema byssoides[1]	**6.20%**	267	AY219839 *Amphinema byssoides*	512/513 (99%)
Amphinema sp.	3.60%	159	AY838271 *Amphinema byssoides*	474/493 (96%)
Atheliaceae 1	3.30%	124	EF493272 *Amphinema byssoides*	431/459 (93%)
Atheliaceae 2	2.20%	46	AY219839 *Amphinema byssoides*	238/249 (95%)
Tylospora asterophora	2.20%	71	AF052556 *Tylospora asterophora*	527/533 (98%)
***Tylospora* sp. 1**[1]	**6.20%**	171	AF052556 *Tylospora asterophora*	506/525 (96%)
Tylospora fibrillosa	1.40%	48	AY010283 *Tylospora fibrillosa*	499/506 (98%)
Tylospora sp. 2	2.50%	38	AF052564 *Tylospora fibrillosa*	479/494 (96%)
Piloderma fallax	1.40%	28	DQ179125 *Piloderma fallax*	539/539 (100%)
Piloderma byssinum	1.40%	24	AY010279 *Piloderma byssinum*	360/362 (99%)
Piloderma olivaceum	1.10%	60	DQ469291 *Piloderma olivaceum*	616/630 (98%)
Pezizales				
Pyronemataceae 1	2.20%	51	DQ069000 *Wilcoxina mikolae*	476/515 (92%)
Pyronemataceae 2	1.10%	14	AY880942 *Wilcoxina mikolae*	417/445 (93%)
Trichophaea hybrida	0.40%	11	DQ200834 *Trichophaea* cf. *hybrida*	554/571 (97%)
Barssia sp.	0.40%	17	AY558743 *Barssia oregonensis*	258/259 (99%)

Table 3.5 cont'd

Final OTU name	% of identified root tips	# of root tips represented	Accession number and name of best NCBI match	# of bases (%match)
Dothideomycetes incertae sedis				
Cenococcum geophilum	1.80%	39	EU427331 *Cenococcum geophilum*	447/448 (99%)
Agaricales				
Cortinariaceae	1.40%	12	DQ097880 *Cortinarius vibratilis*	249/264 (94%)
Cortinarius barlowensis	0.40%	1	EU837212 *Cortinarius barlowensis*	601/602 (99%)
Cortinarius alboviolaceus	0.40%	47	EU821675 *Cortinarius alboviolaceus*	596/600 (99%)
Cortinarius biformis	0.40%	1	AY669688 *Cortinarius biformis*	503/517 (97%)
Cortinarius caperatus	0.70%	15	DQ367911 *Cortinarius caperatus*	605/607 (99%)
Cortinarius salor	0.40%	15	FJ717513 *Cortinarius salor*	577/591 (97%)
Cortinarius sp. 1	0.40%	7	EF077495 *Cortinarius croceus*	436/447 (97%)
Cortinarius sp. 2	0.40%	1	AY669677 *Cortinarius fulvoconicus*	371/376 (98%)
Dermocybe sp.	0.70%	15	DQ481911 *Dermocybe aurantiobasis*	596/596 (100%)
Laccaria bicolor	0.70%	28	DQ367906 *Laccaria bicolor*	605/606 (99%)
Russulales				
Russula laricina	1.40%	25	AY061685 *Russula laricina*	559/568 (98%)
Russula sp. 1	0.40%	13	AY061668 *Russula curtipes*	535/575 (93%)
Russula sp. 2	2.20%	29	AF418612 *Russula aeruginea*	551/579 (95%)
Russula sp. 3	1.10%	18	AF418621 *Russula raoultii*	589/630 (93%)
Russulaceae	0.40%	2	AF540385 *Russula xerampelina*	324/357 (90%)
Lactarius salmonicolor	0.70%	7	AF140265 *Lactarius salmonicolor*	676/698 (97%)
Lactarius deliciosus	0.40%	4	EF685056 *Lactarius deliciosus* var	631/633 (99%)
Sebacinales				
Sebacina sp.	1.40%	63	AF202728 *Sebacina vermifera*	169/170 (99%)
Boletales				
Rhizopogon subbadius	0.40%	1	AF377151 *Rhizopogon subbadius*	574/576 (99%)
Helotiales				
Meliniomyces variabilis[2]	5.10%	135	EF093173 *Meliniomyces variabilis*	520/529 (98%)

Table 3.5 cont'd

Final OTU name	% of identified root tips	# of root tips represented	Accession number and name of best NCBI match	# of bases (%match)
Helotiales				
Cadophora finlandica[3]	1.40%	75	AF486119 *Cadophora finlandica*	453/454 (99%)
Known or suspected mycorrhizal group				
Phialocephala fortinii[4]	1.10%	42	AF214579 *Phialocephala fortinii*	469/470 (99%)
Gyromitra sp.[4]	0.40%	17	AJ544209 *Gyromitra esculenta*	168/170 (98%)
Morchellaceae[4]	0.70%	8	DQ355921 *Morchella rufobrunnea*	153/157 (97%)
Rhizoscyphus ericae[5]	0.70%	19	AM084704 *Rhizoscyphus ericae*	421/433 (97%)
Helotiaceae 1[5]	0.70%	4	EF658755 *Rhizoscyphus ericae*	223/234 (95%)
Helotiaceae 2[5]	0.40%	3	AY789374 *Cudoniella clavus*	419/453 (92%)
Helotiales 1[3]	0.40%	8	FJ000380 *Articulospora tetracladia*	379/459 (82%)
Helotiales 2[3]	1.10%	4	AY853217 *Naevala minutissima*	256/263 (97%)
Helotiales 3[3]	0.40%	4	AB041243 *Allantophomopsis lycopodina*	212/220 (96%)
Helotiales 4[3]	0.40%	17	AF141168 *Scleropezicula alnicola*	241/277 (87%)
Agaricales 1[4]	0.40%	5	DQ486690 *Alloclavaria purpurea*	472/529 (89%)
Agaricales 2[3]	0.70%	9	EF530939 *Mycena rubromarginata*	232/258 (89%)
Basidiomycota[3]	0.40%	25	U85797 *Athelia decipiens*	157/189 (83%)
Ascomycota[3]	0.40%	21	AY606312 *Leptodontidium orchidicola*	377/434 (86%)
Zygomycota[3]	0.40%	24	AJ878780 *Mortierella hyalina*	273/347 (78%)
Known saprotrophs				
Gyoerffyella rotula	4.00%	139	AY729937 *Gyoerffyella rotula*	432/440 (98%)
Chalara sp.	0.40%	2	DQ093752 *Chalara microchona*	450/466 (96%)
Hyaloscyphaceae	0.40%	52	DQ227263 *Hyphodiscus hymeniophilus*	185/190 (97%)
Lasiosphaeriaceae	0.40%	5	EU781677 *Fimetariella rabenhorstii*	430/458 (93%)

[1]The top three most abundant ECM taxa are highlighted by bold type.
[2]ECM genus; species not yet characterized; may form ericoid mycorrhizae (ErM).
[3]Species or group forms ECM and ErM.
[4]Species or group has demonstrated mycorrhizal characteristics.
[5]Species or group forms ErM.

3.3.4 Fungal community richness and evenness

Coleman rarefaction curves of all fungal taxa successfully identified on seedlings sampled from all microsites at all plots approached an asymptote (Figure 3.3). Estimated overall taxon richness was 51.48; SD = 1.66 (Chao1) and 66.69; SD = 4.6 (Jack1) across the site. Separate Coleman curves for each plot treatment (Figure 3.4a) and for each type of microsite (Figure 3.4b) were also asymptotic. Fungal taxon richness appeared to be higher in forest plots than clearcut plots, and lowest in decayed wood microsites.

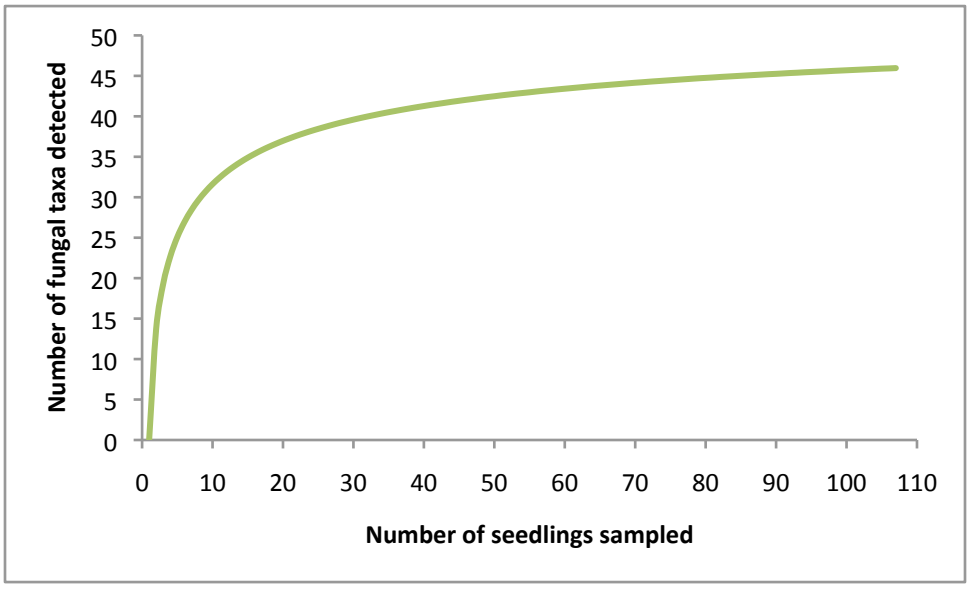
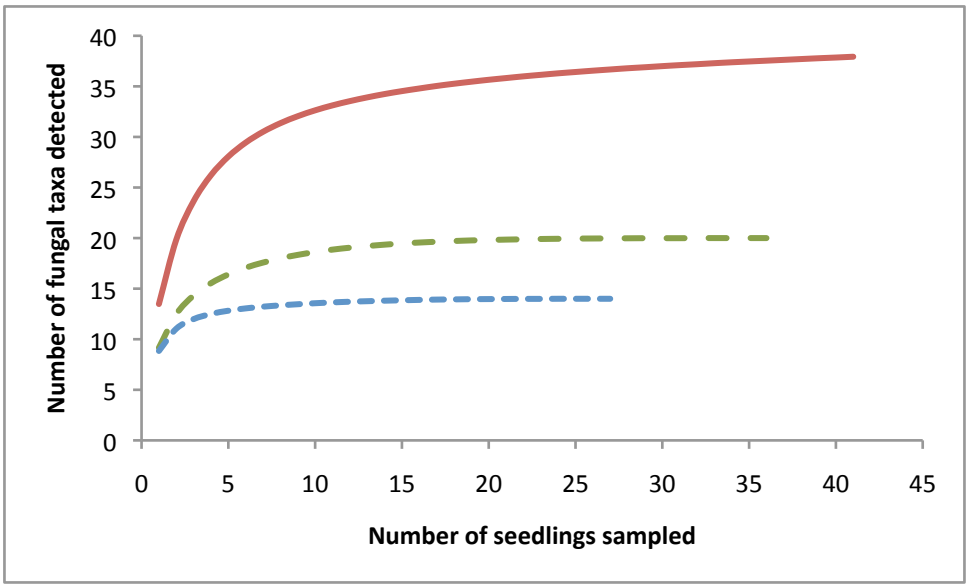


Figure 3.3 Coleman rarefaction curve of all fungal taxa detected on non-mycorrhizal bioassay seedlings sampled for community analysis one year after planting in all microsites at forest and clearcut plots at Sicamous Creek.

a)



b)

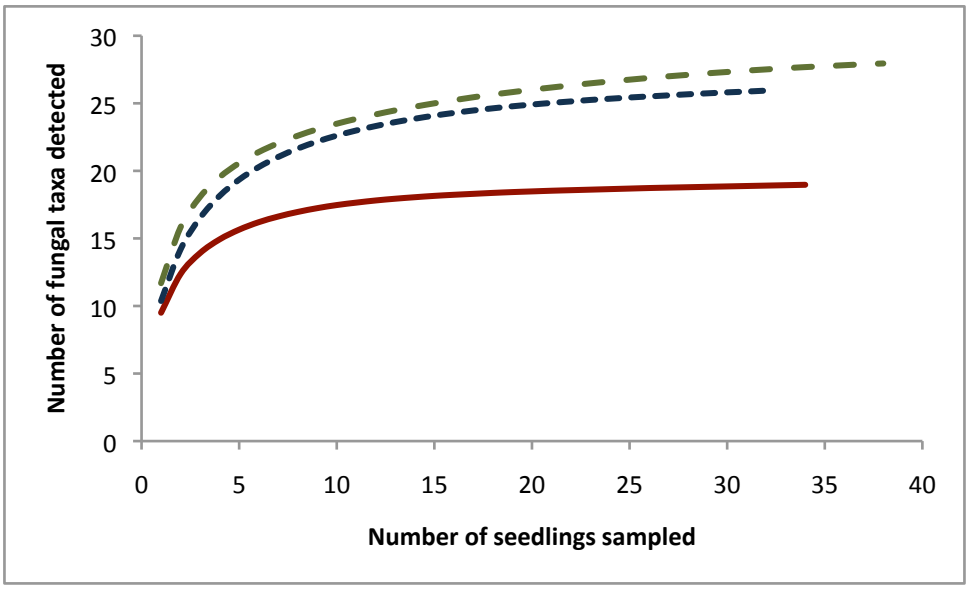


Figure 3.4 Rarefied taxon richness at a) all plots and b) all microsites.
In a), the red solid line represents forest plots, the green dashed line removal plots, and the blue dotted line retention plots. In b), the red solid line represents decayed wood microsites, the green dashed line control soil microsites, and the blue dotted line downed wood microsites.

Microsite did not affect rarefied observed and estimated taxon richness, and taxon evenness per seedling per microsite in each plot treatment, or per microsite per block (i.e., summed across seedlings and plot treatments) (Table

3.6). Rarefied observed and estimated taxon richness, and taxon evenness per seedling were significantly higher at forest plots than at clearcut plots. When considered at the whole plot level only estimated richness and Simpson evenness per plot differed among plot treatments (Table 3.6; Table B.3).

Table 3.6 Rarefied observed (Sobs and Coleman) and estimated (Chao1 and Jacknife 1 estimators) taxon richness and taxon evenness (Shannon and Simpson indices). Values represent means (SD). Letters following numbers in each column indicate significant differences at $p \leq 0.10$ according to a post-hoc Bonferroni test. Bold type is for emphasis only.

			Sobs	Coleman	Chao1	Jack1	Shannon	Simpson
Mean per seedling[1]	Forest	Control	7.0 (1.0)	6.8 (1.2)	6.8 (2.3)	9.6 (3.4)	1.3 (0.4)	3.1 (1.1)
		Downed	7.3 (1.2)	7.2 (1.1)	7.5 (2.5)	10.4 (2.9)	1.6 (0.3)	4.5 (1.5)
		Decay	8.0 (1.0)	7.8 (0.9)	7.1 (1.4)	11.0 (2.9)	1.6 (0.3)	4.8 (1.1)
	Retention	Control	3.3 (2.3)	3.3 (2.2)	2.6 (1.9)	3.6 (2.9)	0.5 (0.6)	1.8 (1.0)
		Downed	2.0 (1.7)	2.0 (1.7)	1.8 (1.4)	2.3 (2.2)	0.9 (0.1)	1.3 (0.5)
		Decay	2.3 (0.6)	2.3 (0.6)	3.8 (1.9)	5.3 (3.1)	1.1 (0.4)	3.0 (1.1)
	Removal	Control	3.3 (1.2)	3.3 (1.1)	4.0 (0.8)	5.8 (0.9)	1.0 (0.07)	2.6 (0.2)
		Downed	4.7 (3.8)	4.7 (3.8)	4.8 (1.8)	6.7 (2.5)	1.1 (0.4)	3.1 (1.4)
		Decay	3.3 (2.3)	3.3 (2.3)	3.1 (1.3)	4.0 (2.1)	0.8 (0.2)	2.1 (0.3)
	p (microsite)[3]		0.80	0.83	0.69	0.64	0.31	0.13
	Forest		**7.4 (1.0) a**	**7.3 (1.0) a**	**7.2 (1.8) a**	**10.3 (2.7) a**	**1.5 (0.3) a**	**4.1 (1.3) a**
	Retention		**2.6 (1.6) b**	**2.5 (1.5)b**	**2.7 (1.7) b**	**3.7 (2.7) b**	**0.8 (0.4) b**	**2.0 (1.1) b**
	Removal		**3.8 (2.4) b**	**3.8 (2.4) b**	**4.0 (1.4) b**	**5.5 (2.1) b**	**1.0 (0.3) b**	**2.6 (0.9) b**
	p (plot)[3]		**<0.0001**	**<0.0001**	**0.0001**	**0.0002**	**0.002**	**0.001**
			Sobs	Coleman	Chao1	Jack1	Shannon	Simpson
Mean per plot or per microsite[2]	Control		12.0 (1.0)	11.9 (1.0)	12.1 (1.3)	16.6 (0.8)	1.8 (0.08)	4.8 (0.4)
	Downed		12.3 (2.3)	12.2 (2.3)	11.7 (4.3)	16.3 (5.5)	1.9 (0.4)	5.4 (2.1)
	Decay		9.7 (0.6)	9.7 (0.6)	10.8 (2.1)	15.2 (3.0)	1.9 (0.3)	5.6 (1.9)
	p (microsite)[4]		0.21	0.22	0.88	0.90	0.94	0.86
	Forest		**16.7 (2.5)**	**16.5 (2.6)**	**17.4 (0.8) A**	**23.7 (2.8) A**	**2.3 (0.1)**	**8.0 (0.9) A**
	Retention		**7.0 (4.4)**	**6.9 (4.3)**	**6.4 (2.8) B**	**9.0 (4.3) B**	**1.3 (0.6)**	**3.6 (1.7) B**
	Removal		**8.0 (2.6)**	**8.0 (2.6)**	**8.9 (2.7) B**	**12.3 (3.7) AB**	**1.6 (0.2)**	**3.8 (0.6) B**
	p (plot)[4]		**0.06**	**0.06**	**0.005**	**0.02**	**0.05**	**0.02**

[1]For each microsite within a plot n=5; for each plot n=15.
[2]For each microsite and each plot, n=3.
[3]p-value is based on a hierarchical ANOVA with microsite nested in plot.
[4]p-value is based on main effects (one-way) ANOVAs.

3.3.5 Fungal community composition

The ECM fungal community on seedling root tips was structured more by plot treatment than by microsite. This was reflected in NMS ordinations, in which the forest samples clustered separately from clearcut samples (Figure 3.5). I also explored this dataset with PCA, but this resulted in a distorted, clumped community ordination that explained almost no (1.5 % for relative abundance) or only a moderate amount (55.4 % for presence-absence) of the variation, and provided little insight into the relationships among taxa, plots and environmental variables (not shown). According to MANOVA analysis, ECM communities varied among plot treatments when analysed based on relative abundance ($p=0.050$; Table 3.7a).

There were several dominant fungi that differed overall among plots based on univariate tests of their relative abundance: *Thelephora terrestris* ($p\leq0.001$; Table B.4), *Amphinema byssoides* ($p=0.02$), *Tylospora* sp. 1 ($p=0.03$), *Tylospora fibrillosa* ($p=0.02$), and *Tylospora* sp. 2 ($p\leq0.05$). Post-hoc Bonferroni tests showed that *Thelephora terrestris* was more abundant in both types of clearcut plots (removal $p=0.001$; retention $p<0.0001$) than it was in forest plots; it was also an indicator species for retention plots ($p=0.003$). *Amphinema byssoides*, was more abundant in removal than in retention plots ($p=0.02$), and was an indicator species for the removal treatment ($p=0.06$). *Tylospora* sp. 1 occurred more often in forest plots than in removal plots ($p=0.050$), while *Tylospora* sp. 2 ($p=0.04$) and

Tylospora fibrillosa (p=0.02) occurred only in forest plots, and were both indicator species for forest plots (p≤0.05).

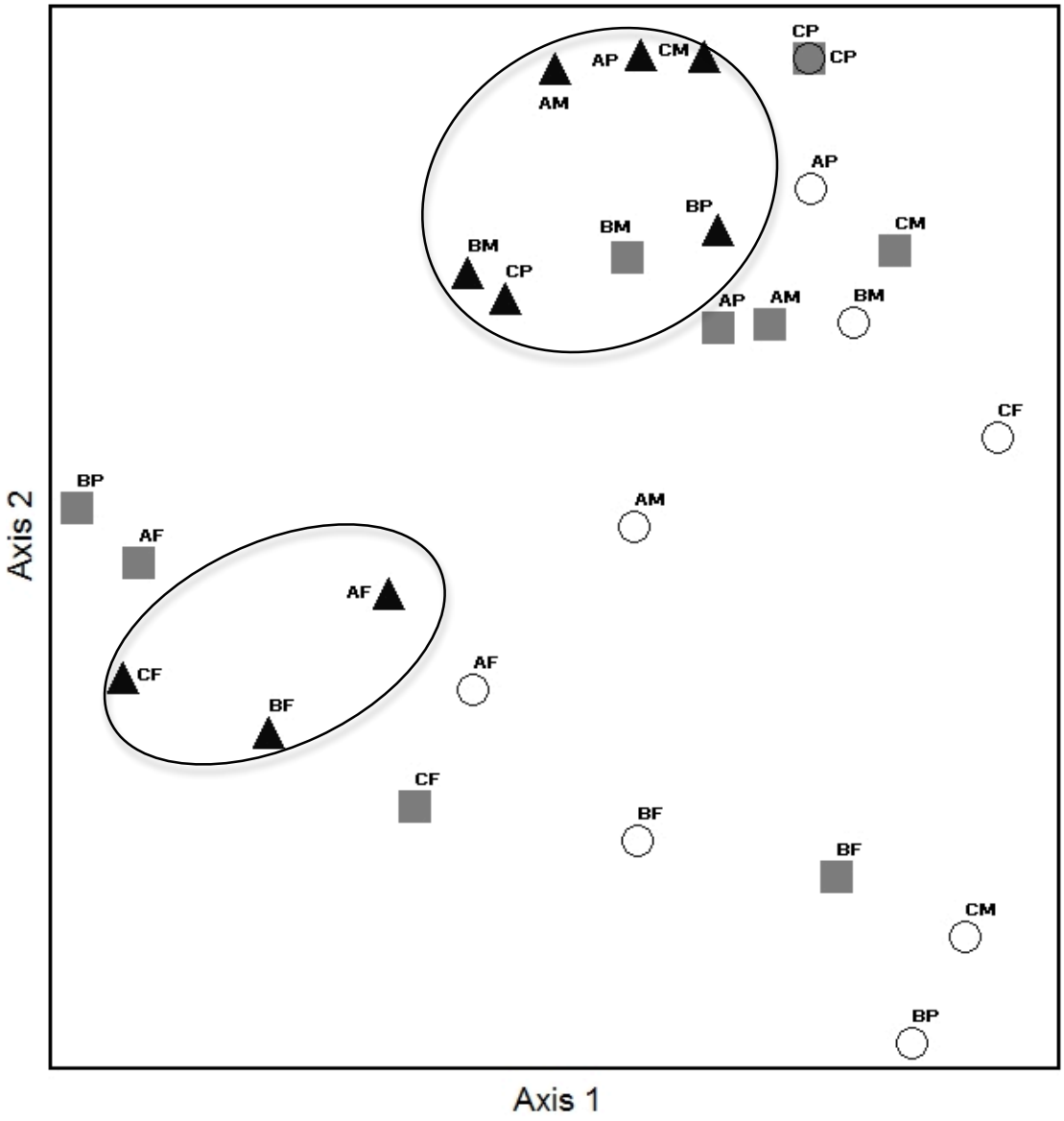
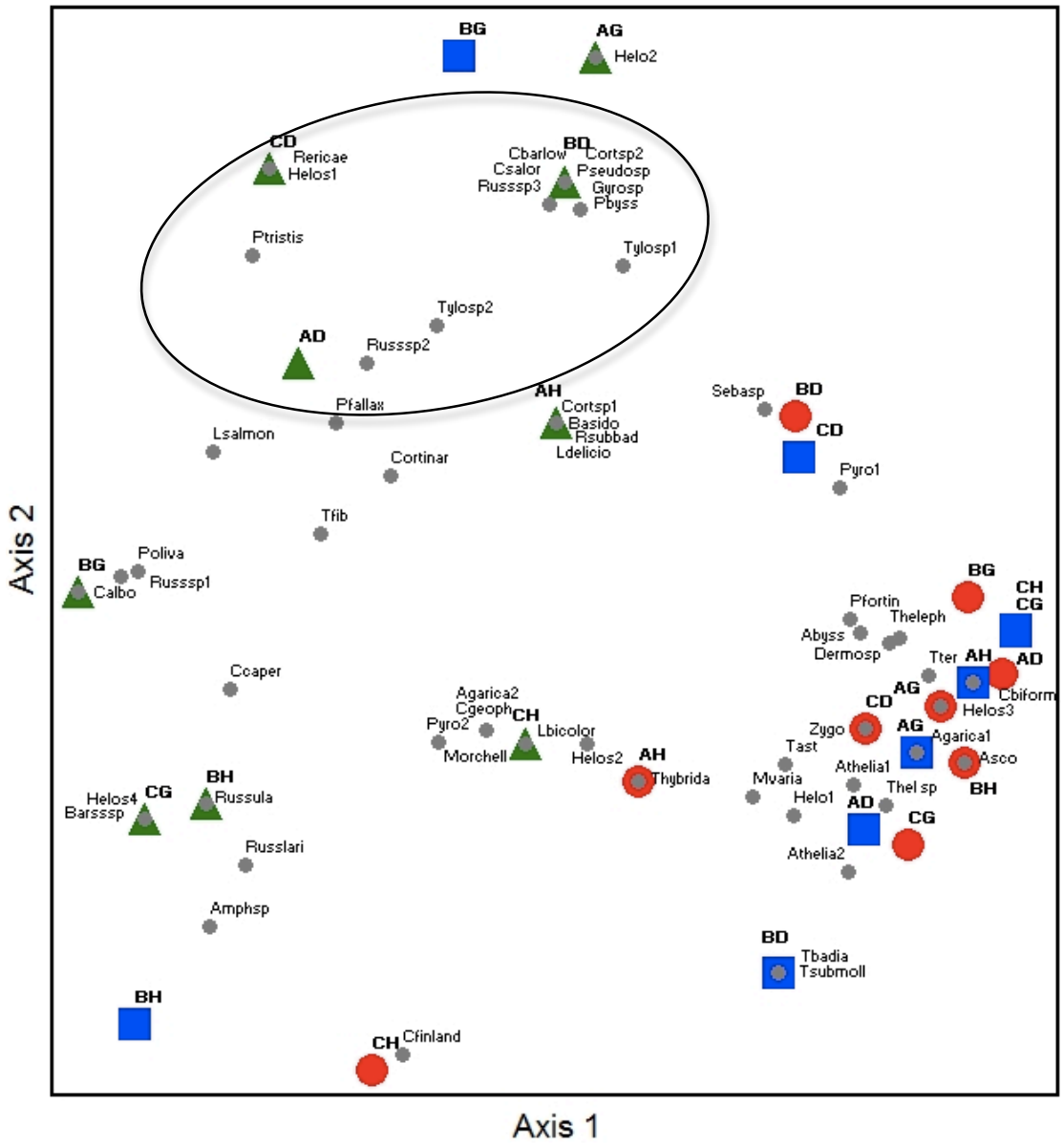


Figure 3.5a NMS ordinations of root tip presence-absence showing the relationship among blocks (A, B, and C), plots, and microsites.
Clustering of decayed wood microsites (discussed in the text) are outlined by black circles. Microsite icons are highlighted as control soil (grey squares), downed wood (open circles), and decayed wood (black triangles), and plots are forest (F), retention (P), and removal (M). Stress was fair for this two-dimensional solution (16.2), with axis 1 explaining 21.8% of the variation, and axis 2 explaining 41.1%.



.

Figure 3.5b NMS ordinations of root tip relative abundance showing the relationship among blocks (A, B, and C), plots, and microsites.
Clustering of decayed wood microsites (discussed in the text) are outlined by black circles. Plot icons are highlighted as forest (green triangles), retention (blue squares), and removal (red circles), and microsites are control soil (G), downed wood (H), and decayed wood (D). Stress is good in this three-dimensional solution (14.1), with axis 1 explaining 37.1% of the variation, while axis 2 explained 20.6%, and 3 explained 18.3.

Table 3.7 Multivariate hierarchical ANOVA of a) overall ECM community relative abundance, b) ECM community relative abundance in clearcut plots, and c) main effects ANOVA of ECM community relative abundance in forest plots.

a)

	Test	Value	F	Effect	Error	p
Intercept	Wilks	0.002	412.973	56	41	0.000
Block	Wilks	0.170	1.043	112	82	0.424
Plot treatment	**Wilks**	**0.117**	**1.412**	**112**	**82**	**0.050**
Microsite(Plot)	Wilks	0.007	0.999	336	253.9	0.506

b)

	Test	Value	F	Effect	Error	p
Intercept	Wilks	0.003	363.2	30	28	0.000
Block	Wilks	0.192	1.20	60	56	0.247
Plot treatment	Wilks	0.443	1.18	30	28	0.335
Microsite(Plot)	Wilks	0.049	1.08	120	113.922	0.344

c)

	Test	Value	F	Effect	Error	p
Intercept	Wilks	0.0003	222.0	36	2	0.004
Block	Wilks	0.000006	23.6	72	4	0.003
Microsite	Wilks	0.0005	2.45	72	4	0.198

Decayed wood microsites clustered together both within forest plots and within clearcut plots in ordinations using presence/absence data (Figure 3.5a). When ordinated using relative abundance data, the decayed wood microsites in the forest plots remained close to each other; however, the decayed wood microsites in the clearcut plots were no more aligned with each other than they were with any other microsite. Furthermore, in neither ordination were decayed wood microsites in clearcut plots more closely associated with forest samples than they were to control microsites in their respective plots. When I examined these data with a hierarchical MANOVA, ECM fungal community composition did not differ significantly among microsites based on relative abundance (p=0.5; Table 3.7a).

The relative abundance of three species varied overall among microsites when I analysed ECM taxa independently with univariate ANOVA: *Cortinarius biformis* ($p=0.04$), *Dermocybe* sp. ($p=0.04$), and *Meliniomyces variabilis* ($p=0.03$). Specifically, in retention plots, *Cortinarius biformis* and *Dermocybe* sp. were present only in downed wood microsites, and *Meliniomyces variabilis* was present only in decayed wood. With the exception of one other taxon that was detected adjacent to downed wood in a removal plot, *Dermocybe* sp., and other members of the Cortinariaceae were otherwise found only in forest plots (where they were represented in all microsites and at all blocks). *M. variabilis* was also only otherwise found in forest plots; I detected it in decayed wood and control soils at all blocks.

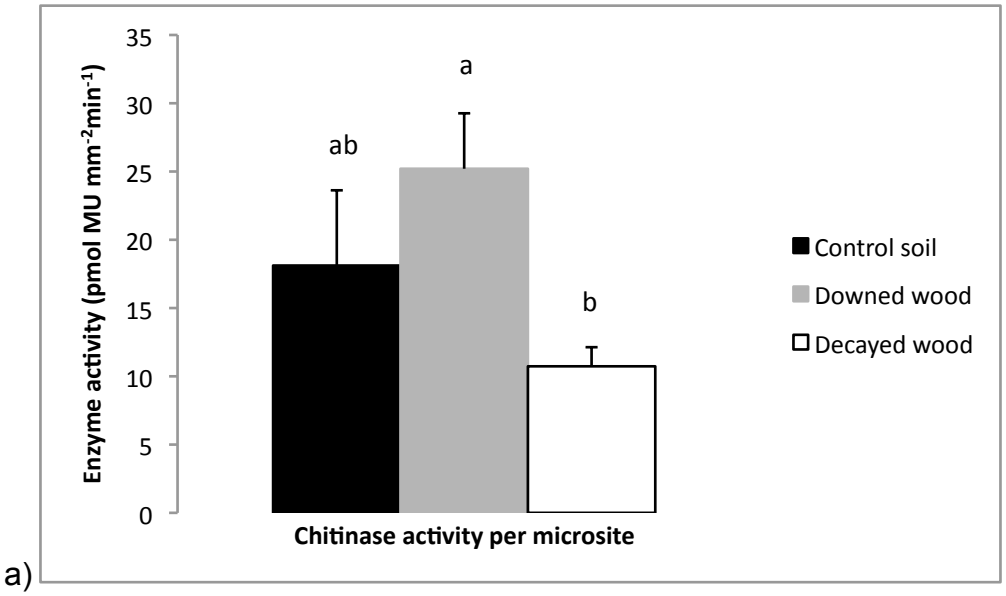
I also performed a MANOVA analysis on forest plots and clearcut plots separately because of the clear distinction between forest and clearcut plots (Table 3.7 b and c). This analysis confirmed that retention and removal plots were not different from each other ($p=0.34$; Table 3.7b), but there were no significant differences in ECM community composition among microsites. The relative abundance of *C. biformis*, *Dermocybe* sp., and *M. variabilis* remained significantly different overall among microsites in clearcuts only ($p \leq 0.10$).

3.3.6 Enzyme activity of individual ectomycorrhizal taxa in microsites and plots

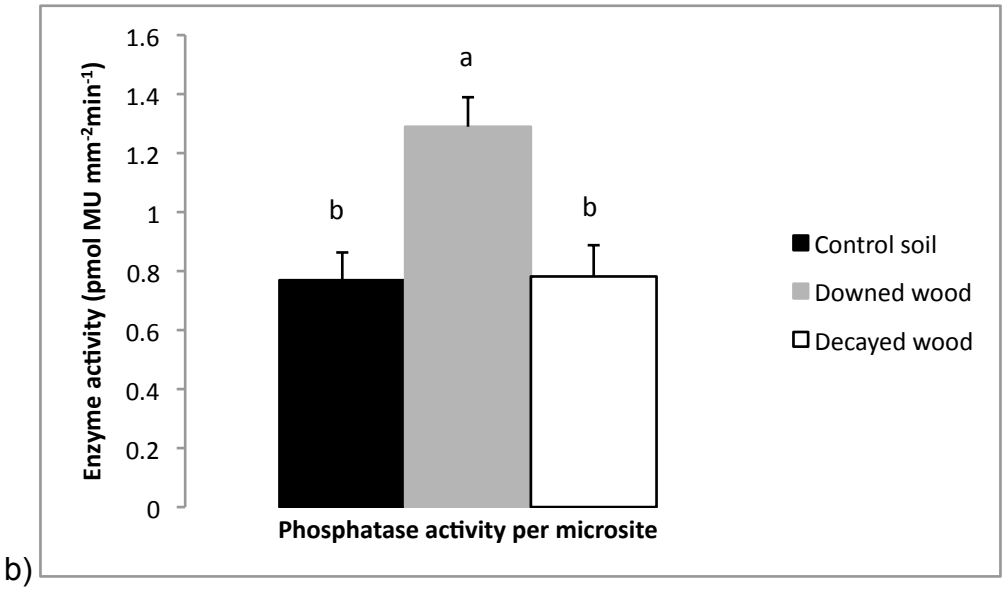
I proceeded to test the enzyme profiles of individual taxa for which I had sufficient replication, and this revealed apparent plasticity among microsites for Tylospora spp. overall. *Amphinema byssoides* and *Wilcoxina* spp. (a combination of Pyronemataceae 1 and 2, which are very likely to both be *Wilcoxina* spp.), mycorrhizae were frequent and abundant enough to test for phenotypic plasticity by determining whether their enzyme profiles varied among plot treatments and microsites. *T. terrestris* was encountered less frequently in forest plots, so I tested it only in clearcut plots. *Tylospora* spp. (a combination of *T. asterophora, Tylospora* sp. 1, *T. fibrillosa*, and *Tylospora* sp. 2) was not frequent in clearcut plots, therefore I could only test for differences among microsites in forest plots. The overall enzyme profile of *Tylospora* spp. differed significantly among microsites in the forest (p=0.02) (Table 3.8). The activities of chitinase (p=0.02) and phosphatase (p=0.01) differed overall among microsites for *Tylospora* spp. in forest plots, and were highest adjacent to downed wood (Figure 3.6). No significant differences were detected in overall enzyme profiles for any other taxa among plots or microsites.

Table 3.8 Multivariate hierarchical ANOVA of the enzyme activity profile (i.e. the activity of eight enzymes per seedling) of *Tylospora* spp. among microsites in forest plots. N=9.

	Test	Value	F	Effect	Error	p
Intercept	Wilks	0.003	43.4	8	10	0.00
Block	Wilks	0.227	1.14	16	20	0.248
Microsite	Wilks	0.109	2.55	16	20	0.025



a)



b)

Figure 3.6 a) Chitinase and b) phosphatase activity of *Tylospora* spp. mycorrhizae among control soil, downed and decayed wood microsites in forest plots.
Columns with error bars represent raw means +SEM, and letters above each column show significant differences at $p \leq 0.10$ based on post-hoc Bonferroni tests. All statistical tests are based on log-transformed data. N = 9 seedlings per microsite.

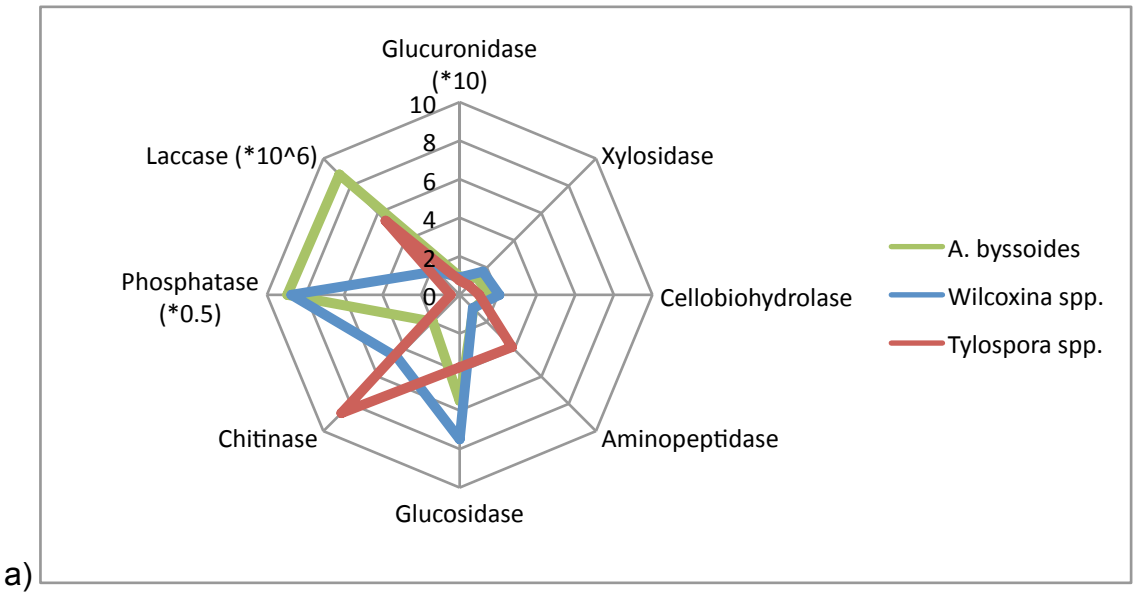
3.3.7 Functional complementarity among individual ectomycorrhizal taxa

I aimed to evaluate if and how the enzyme activity profiles varied among dominant taxa, with the specific objective of determining if this subset of the community in microsites and plots exhibited evidence of functional complementarity. However, there were no taxa with sufficient replication at the microsite scale such that I could compare their enzyme activities to each other within all microsites. I did test these differences among forest and clearcut plots.

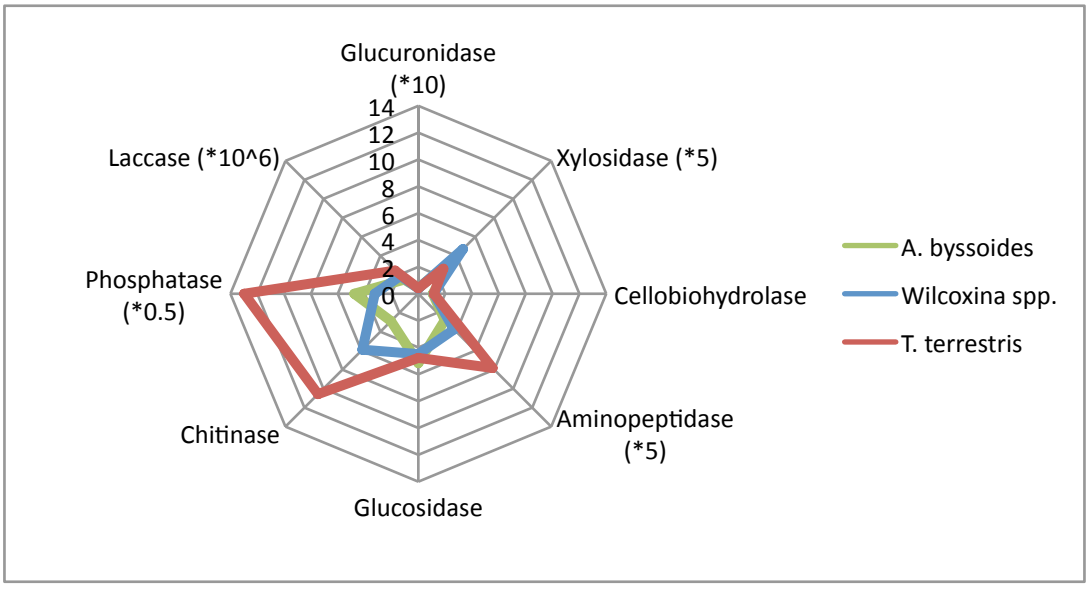
Polar graphs of the individual enzyme profiles of select taxa (i.e. using all available data for each taxon as we did in section 3.3.6) in clearcut and forest plots suggested that functional complementarity was occurring among taxa within plot types (Figure 3.7). I tested this observation using a multivariate ANOVA, with all eight enzymes as response variables, taxa as random factors, and with n=3 observations (one from each microsite type) per plot per taxon. Enzyme activity profiles differed overall among *Tylospora* spp., *A. byssoides,* and *Wilcoxina* spp. mycorrhizae in forest plots ($p=0.026$; Table B.5a and Figure 3.7a), and among *T. terrestris*, *A. byssoides,* and *Wilcoxina* spp. mycorrhizae in clearcut plots (retention and removal plots combined; $p<0.0001$; Figure 3.7b).

There were specific differences among all three or between two taxa for individual enzymes (please refer to Figure 3.7a). In forest plots, *Tylospora* spp. aminopeptidase activity was significantly higher than that of *A. byssoides* and

Wilcoxina spp. (both $p<0.001$; Table B.5 b and c), and *Tylospora* spp. chitinase activity was significantly higher than that of *A. byssoides* ($p=0.025$). *Tylospora spp.* phosphatase activity was significantly lower than that of both of *A. byssoides* and *Wilcoxina* spp. (both $p<0.001$), while *Wilcoxina* spp. laccase activity was significantly lower than that of *A. byssoides* and *Tylospora* spp. (both $p<0.05$). *Wilcoxina* spp. glucosidase and cellobiohydrolase activity were significantly higher than that of Tylospora spp. (both $p<0.05$). There were also specific differences in enzyme activity among three or between two taxa, in clearcut plots combined (please refer to Figure 3.7b): aminopeptidase, chitinase, phosphatase, and laccase activities of *T. terrestris* mycorrhizae were significantly higher, on a surface-area basis, than those of both *Wilcoxina* spp. and *A. byssoides* (all $p<0.05$), and *Wilcoxina* spp. xylosidase activity was significantly higher than that of *T. terrestris* ($p=0.033$).



a)



b)

Figure 3.7 Overall enzyme profiles for ectomycorrhizae formed by a) *A. byssoides, Wilcoxina* spp., and *Tylospora* spp. in forest plots, and b) *A. byssoides, Wilcoxina* spp., and *T. terrestris* in clearcut plots.
Polar graphs were constructed for each taxon using n=3-5 seedlings per microsite per plot. Enzyme activity is measured in pmol $mm^{-2}min^{-1}$ surface area for all enzymes but laccase (mol $mm^{-2}min^{-1}$). Some of these raw values have been scaled so that all enzymes can fit on the graph; these enzymes are followed by the multiplication factor in brackets.

3.4 Discussion

3.4.1 Coarse woody debris provided habitat for ECM fungi in clearcuts

I expected that over the medium and long term, coarse woody debris would influence the development of ECM fungal communities in clearcuts by: (i) providing suitable habitat for some ECM fungi and/or (ii) retaining species of ECM fungi from the harvested stand. I predicted that if downed and decayed wood were helping to retain ECM fungi from the original forest stand, I would detect forest ECM fungi in these microsites. I anticipated that the abiotic properties of the soil in woody microsites would be similar to those in the forest soil, creating favourable habitat for retained ECM fungi or for newly recruited ECM fungal taxa. I found support for both these predictions: woody microsites in the clearcuts had similar soil attributes to the forest, and forest ECM fungal species were detected in these microsites in the clearcuts.

Fourteen years post-harvest, I found that nutrient availability was generally lower in clearcut plots than forest plots, but that downed and decayed wood microsites within the clearcuts had nutrient levels most similar to those of forest soils. In addition, pH in downed and decayed wood microsites was more similar to that of forest soils. Furthermore, microsites beside intact downed wood on clearcuts were cooler and moister than other clearcut soils and this made them more similar to microclimates in forests. The differences in soil properties that I found between clearcut and forest soils are comparable to earlier studies at Sicamous

Creek (Hannam and Prescott 2003; Prescott et al., 2003; Redding et al., 2003) as well as to those from other northern coniferous forests (Grønflaten et al., 2008), and many other temperate forest types (Berthrong et al., 2011). In one of the few recent studies to explicitly examine the effect of retaining post-harvest residue on soil chemistry, Wall (2008) found a short-term retention of soil N levels in spruce forests, but Jones et al. (2008) found similar initial effects to be short-lived in pine plantations (i.e. they were no longer detectable in fewer than 16 yrs. post-harvest, which is a similar timeframe for the clearcuts we studied at Sicamous Creek). Many older studies of CWD retention focused on rates of log decomposition in clearcuts (Mattson et al., 1987; Marra and Edmonds, 1996), and some have assessed subsequent organic input to the soil (Yanai et al., 2003, and references therein). Studies that have attempted to measure overall changes in the soil chemistry below downed wood did so in old or undisturbed systems (Kayahara et al., 1996; Laiho and Prescott, 1999; Spears et al., 2003; Spears and Lajtha, 2004). My findings suggest that retention of post-harvest woody debris does not change plot-scale edaphic conditions; however, it is clear from this study that retention of large downed wood and its subsequent decay can provide microsites that create forest-like habitat inside drier, hotter, and nutrient-depleted clearcut plots. These observations support my first proposed mechanism for the influence of CWD retention on ECM fungal communities in high elevation spruce forests: that woody microsites provide suitable habitat for ECM fungi.

The soil abiotic properties discussed above are known to help shape ECM communities (Molina et al., 2008). The microclimates in the woody microsites in the clearcuts were similar to conditions in the forest, but they did not support ECM fungal communities in the clearcuts that were similar to those in the forest. The ordinations and multivariate analyses indicated that ECM fungal communities in decayed or downed wood microsites in clearcuts were no more similar to forest communities than the control microsites were to the forest communities. I also acknowledge that collectively, the ECM fungal community did not co-vary with microsite conditions. Nevertheless, examination of individual species revealed evidence that the woody microsites may have retained, or supported colonization by, fungi that were otherwise found only in forest samples. In particular, *Cortinarius biformis* and *Dermocybe* sp. were present only adjacent to downed wood, and *Meliniomyces variabilis* was present only in decayed wood, in retention plots of clearcuts. I expected to find an overall difference in ECM community composition among microsites, since it has been shown that several ECM fungal species, including *Meliniomyces bicolor*, prefer woody microsites (Rajala et al., 2011), and that these species tend to also prefer the undisturbed forest floor (Tedersoo et al., 2008). Tedersoo et al. (2008) also found *Cortinarius* spp. almost exclusively in the forest floor, with only one species detected on spruce roots associated with logs, and, in beech forests, Dickie et al. (2009) found only one member of the Cortinariaceae to inhabit both forest and clearcut sites. In general, it would appear that forest habitat is important for the majority of *Cortinarius* spp. My findings are consistent with these previous

studies, and they lend support for the idea that decayed and downed wood microsites in clearcuts provide medium- and long-term habitat for certain species of ECM fungi (Molina et al., 2008; 2010), however, I remain surprised that some of the more abundant forest taxa were not more strongly aligned with woody microsites. Nevertheless, I did detect forest ECM fungi in woody microsites that were not present in the control microsites within the clearcuts, which provides evidence for our second mechanism: that CWD retention can help to retain ECM species from the previous forest stand.

I cannot determine whether the occurrence of particular species in woody microsites in the clearcuts at Sicamous Creek was attributable to persistence since logging or recolonization. Both factors may have been important. Rajala et al. (2011) suggest that the colonization of decayed logs by ECM fungi may be a vital intermediate step in seedling reestablishment, although fungal types that fruit on the underside of decaying logs may not necessarily have a colonization advantage (Tedersoo et al., 2009). This might explain why I did not detect an affinity for woody sites by *Amphinema* and *Tylospora* spp. Most forest ECM fungi may not be well suited to the altered abiotic environment of a clearcut in the short term (Jones et al., 2003), and many perish within a few years of harvest (Hagerman et al., 1999). The availability of woody microsites may hasten recolonization of the clearcut by fungi that are otherwise limited to dispersal in from the original forest (Peay et al., 2010) by providing habitat in which ectomycorrhizae on dying root systems either in the decayed wood or in the soil

below downed wood can exist long enough to colonize newly planted or naturally regenerating roots.

3.4.2 ECM community enzyme activity varied according to plot treatment

Differences in ECM fungal community composition and in soil abiotic properties between forests and clearcut plots were reflected in overall enzyme activity, which also varied most at the plot scale. At this scale, community enzyme activity appeared to be related to substrate availability, for example, laccase activity was higher in forest plots where total C was highest, and cellobiohydrolase and aminopeptidase activity were lower at retention plots where total C and total N was lowest. Geisseler and Horwath (2008) showed that microbial protease (e.g. aminopeptidase) activity was substrate-driven, specifically that protease activity increased when organic N was abundant, and that protease synthesis was limited by the availability of C, and therefore also driven by its absence. Burke et al. (2011) confirmed that extracellular hydrolytic enzymes involved in N, C, and P degradation (specifically chitinase, cellobiohydrolase, glucosidase, xylosidase, and phosphatase) were spatially correlated with ECM/saprobe communities, but this was in turn only correlated with higher mineral N. Jones et al. (In review) found that mycorrhizosphere activities of the same eight enzymes we analysed were positively correlated with total N, and that the enzymes activities of some taxa were negatively correlated with pH. In my study, phosphatase was the only enzyme (in addition to laccase) whose activity was highest in forest plots where

total N was highest, and only cellobiohydrolase and phosphatase appeared to be affected by pH: activity was lowest in retention plots where pH was highest. Ectomycorrhizal community laccase activity was positively correlated with water potential and negatively correlated with temperature in an oak forest (Courty et al., 2010), which may explain my finding of elevated laccase activity in forest plots. My observation of an association between substrate availability and enzyme activity is potentially confounded by plot-scale differences in ECM fungal community composition.

Enzyme activity did not appear to be driven by which individual taxon was most abundant in each plot. For example, the enzyme pattern of *A. byssoides*, which was most abundant in removal plots, was not reflected in the overall activity in removal plots. Also, ECM fungal taxa did not demonstrate the highest of enzyme activities in the plots where they were most abundant (e.g. the aminopeptidase and phosphatase activity of *A. byssoides* was significantly lower in removal plots than they were in forest plots, and the aminopeptidase and phosphatase activity of *A. byssoides* approached or equaled that of forest plots overall). The absence of a relationship between abundance and functional contribution is consistent with other findings (Rineau and Courty, 2011), and suggests that in my study, dominance in one plot (as interpreted by the relative abundance of one taxon) does not mean that said taxon is particularly well-adapted to exploiting soil nutrients to the exclusion of those with which it co-occurs. Some have found, however, that dominance in one type of substrate (e.g. dead wood, presumably

harbouring abundant saprotrophs) is associated with proportionally higher enzyme activity (e.g. chitinase activity, presumably for degrading said saprotrophs), at least for some taxa (e.g. members of the Thelephoraceae), suggesting adaptation to a particular niche (Buée et al., 2007). In addition, Buée et al. (2007) found that when the same species was sampled from different substrates, a unique pattern of activity related to that substrate was detected; this physiological plasticity was observed for all dominant ECM fungi in their study. I observed that there were differences in community enzyme profiles at the plot level in this study. While this reinforced the dissimilarities between forest and clearcut plots, it did not provide any insight into ECM fungal community plot-level plasticity. This is because the ECM fungal communities differ among the plot types, so I cannot assume that some of the same ECM fungal taxa are actually performing differently in different plots.

I found no evidence of unique patterns of depolymerase activity in specific microsites, and therefore this study cannot offer support for strong niche development with respect to the breakdown of soil macromolecules and the release of soluble nutrients as a feature of the entire ECM community at the microsite scale. I expected that if ECM communities did not vary with microsite, I would observe physiological plasticity in dominant taxa in response to changes in substrate availability in the different microsites. I observed patterns consistent with this prediction for the dominant forest taxon *Tylospora* spp., whose chitinase and phosphatase activities differed overall among microsites. I did not detect

physiological plasticity for ECM fungal species that dominated the clearcuts, nor could I conclude that any ECM taxa demonstrated plot-level plasticity.

3.4.3 Dominant ECM species demonstrated functional complementarity

I expected that if little physiological plasticity was observed for individual ECM fungal species, co-occurring species would show different and complementary patterns in the same microsite. This would indicate that individual taxa are well-adapted to exploiting nutrients in a specific niche, even though we could not detect this for the entire ECM community overall. I was not able to test physiological differences among taxa in same microsite. Based on individual enzyme profiles however, I speculated that complementarity may be occurring among taxa within plots, and I observed varying degrees of this for three co-occurring taxa in the forest and in the clearcut.

There is growing consensus that enzyme profiles in mycorrhizospheres are heavily influenced by the fungal symbiont (Buée et al., 2007; Jones at al., 2010; In review), and my results are consistent with this conclusion at the plot scale. Furthermore, there has been speculation (Jones et al., 2009) and support (Jones et al., 2010) for greater differences in enzyme profiles among taxa in forests than in disturbed areas. In this study, functional complementarity was indeed most apparent in the undisturbed forest, where the activities of laccase, phosphatase, aminopeptidase, chitinase, glucosidase and cellobiohydrolase differed among

ECM taxa. In the clearcuts, however, where laccase, phosphatase, aminopeptidase, chitinase, and xylosidase also differed among taxa, functional complementarity was difficult to discern because of the overall dominance of most of the enzyme profile by one taxon, *T. terrestris*. Buée et al. (2007) used the same technique as in this study in a temperate oak forest site. They found that different ECM fungi from the same microsite (organic soil, mineral soil and dead wood) had different suites of enzymatic capabilities. These capabilities appeared to complement each other, especially in the undisturbed organic soil. Nevertheless, in their study, there was one taxon, a *Tomentella* sp., whose enzyme profile appeared to overlap that of all the others. This example, along with my finding for *T. terrestris* in the clearcut, is perhaps an extreme case of functional similarity within a community. This is possible if it results in the useful repetition of functional traits, and if the community is taxonomically diverse (Rineau and Courty, 2011). This has enormous relevance because *T. terrestris* was abundant in clearcut plots at Sicamous Creek, and the presence of taxa with such a broad fundamental niche could perhaps buffer the loss of other taxa. This might suggest that although species richness and diversity are limited, and the community structure has changed, some physiological processes in the clearcut are maintained. However, my community enzyme profile data does not support similarity between physiological processes in the forest and clearcut. In addition, the dominance of these processes by one taxon contrasts strongly with the resilience provided by a more functionally diverse community (Rineau and

Courty, 2011), which may be important in disturbed systems such as clearcuts (Jones et al., 2003).

3.4.4 Forest plots were more diverse than clearcut plots

The clear distinction between ECM fungal communities in forest and clearcut plots confirms what many others have found (Ding et al., 2011, and summarized in Jones et al., 2003). *Amphinema byssoides*, *Thelephora terrestris*, and *Tylospora* spp. emerged as the most abundant taxa on seedling roots; *A. byssoides* and *T. terrestris* dominated removal and retention plots, respectively, while *Tylospora* spp. occurred more often in forest plots. These fungi were among the most abundant taxa on sapling roots in previous studies of clearcuts at this site (Walker et al., 2012; Chapter 2). *A. byssoides* and *T. terrestris* are dominant pioneer species, known to be important for seedling regeneration in similar temperate forest systems (Kranabetter, 2004). The clearcut plots at Sicamous Creek site still contain diverse living ECM fungal hyphae (Walker, Chapter 2), as do the forests (Walker, Chapter 4), therefore the differential success of these three dominants may be explained by the exploration pattern of their extramatrical hyphae. *Amphinema* and *Thelephora* are both medium-distance exploration types, which is intermediate among the established categories, and means they form rhizomorphs that can branch and interconnect; *Tylospora* is a short-distance exploration type which does not form rhizomorphs (Agerer, 2001). This is of functional relevance for water and nutrient and

transport in addition to nutrient acquisition, since an increase in surface area could directly improve the absorptive capacity of the fungus (Agerer 2001; Read and Perez-Moreno, 2003). Studies comparing exploration types and levels of nutrient availability have, however, been equivocal. For example, medium-distance exploration types have been found to dominate nutrient rich soils where extensive mycelia should be unnecessary for sufficient nutrient uptake (Heinonsalo et al., 2007; Kranabetter el al., 2009). Others have come to the opposite conclusion regarding exploration types in soil layers (Baier et al., 2006; Courty et al., 2008), and concerning the predominant exploration type in areas of low root density such as clearcuts (Peay et al., 2011; Heinonsalo et al., 2007). In this study, *Amphinema* and *Thelephora* were the most abundant taxa in the clearcuts, where nutrients were limited, and root density was low, while *Tylospora* was most abundant in the forest, where the organic layer remained intact. My results were a mix of the findings reported above, but suggested that ECM fungal communities were unique in forests compared to clearcuts at this site because, in part, longer exploration types were dominant where nutrient availability was lowest, and where roots were widely spaced.

Clearcuts had a unique ECM fungal community as compared to the forest, but two important biotic factors could have also contributed to reduced ECM diversity: the low diversity of ECM hosts (Ding et al., 2011; Tedersoo et al., 2008), and the limited access to photosynthate-carbon for ECM fungi (Druebert et al., 2009; Pena et al., 2010). The seedlings sampled in this study were from

one conifer species (*P. engelmannii*), and, in the clearcuts, their root systems were generally isolated from other woody roots, the majority of which would have been from *P. engelmannii* operationally planted a decade prior (Walker, Chapter 2). Root systems of the *P. engelmannii* seedlings sampled from the forest were surrounded by ECM woody roots, including those of the co-dominant conifer at this site (*Abies lasiocarpa*), and by the roots of ericaceous shrubs (especially *Rhododendron albiflorum*) known to host fungi that form ectomycorrhizae (Read et al., 2004; Grelet et al., 2010). As such, the seedlings in the forest were in very close proximity to many more fungal taxa with potential affinity to them. In addition, the year-old seedlings likely provided very little new photosynthate-C to their fungal symbiont. In the clearcuts, where the alternate hosts were widely-spaced saplings that had not yet reached a high potential for carbon provision to their symbiont (Twieg et al., 2007), this could have selected for ECM fungi that were less C-demanding (Druebert et al., 2009), leading to the loss of rare taxa that require new photosynthate (Pena et al., 2010). Seedlings in the forest, on the other hand, were likely colonized by fungi already attached to a large, mature tree with an abundant source of new carbon. It is likely that these biotic features operate in concert with additional factors to drive the stark contrasts in community diversity between the clearcut and forest plots.

4 The community composition and enzymatic activity of fungal hyphae colonizing decayed wood and mineral soil microsites differs among forest plots

4.1 Synopsis

The extensive hyphal networks of soil fungi play an important role in forest decomposition and nutrient cycling by releasing numerous degradative enzymes and subsequently absorbing soluble molecules (Deacon, 2006). Fungal saprotrophs break down complex forms of dead organic matter, including wood, and most mycorrhizal fungi can provide their host plant with mineral nutrients such as nitrogen (N) and phosphorus (P) (Deacon, 2006). Ectomycorrhizal (ECM) fungi produce phosphatases and laccases, and are capable of degrading hemicellulose and cellulose (Baldrian, 2006; Caldwell, 2005; Leake et al., 2002; Smith and Read, 2008). They also produce chitinases and aminopeptidases, and can assimilate organic and inorganic forms of N (Aerts, 2002; Buée et al., 2007; Carlile et al., 2001; Courty et al., 2007; Deacon, 2006). Although much work has been done on the physiology of ectomycorrhizal root tips (Buée et al., 2007; Courty et al., 2007), enzyme production and nutrient absorption occur primarily in the ECM fungal hyphae (Agerer, 2001; Genney et al., 2006). There is little information on the distribution of ECM mycelia in wood, but previous field studies have shown that ECM hyphae of certain species are found in distinct organic or mineral niches (Genney et al., 2006; Landeweert et al., 2003). However, it is not

known how different substrates colonized by the ECM fungal hyphae, for example, decaying wood on the forest floor, affect their enzyme activity.

This study addresses the role of downed and decayed wood in forest ecosystems. The objective of this study (Objective 5 of the thesis) was to determine whether the composition and physiological activity of soil fungal communities present as hyphae differed among microsites adjacent to hard wood, in decayed wood, or in mineral soil of mature forests at Sicamous Creek. I expected substrate samples to be dominated by ectomycorrhizal (ECM) fungal taxa because I collected them immediately surrounding spruce seedlings planted in the different forest microsite types. I used massively parallel sequencing of DNA extracted from the samples to identify fungal taxa present as mycelia in the substrates, and also tested the activity of eight extracellular depolymerases known to be produced by ECM fungi. This allowed me to compare patterns of enzyme activity among fungal communities in the different substrates, in addition to the enzyme activity of individual ECM taxa present as hyphae in the different microsites types.

> **Hypothesis 5:** Taxonomic and functional differences will be found in the overall fungal community, and in the ECM fungal community among decayed wood, mineral soil, and hard downed wood microsites.

4.2 Methods

4.2.1 Field site description and experimental design

The experimental area examined in this chapter includes three replicate 1 ha plots in the undisturbed forest, each one within a larger (30 ha) experimental unit, located approximately 1 km apart. The site details of these forest plots are as described in the Introduction, and in Chapter 3, Section 3.2.1.

4.2.2 Seedling harvesting and sample processing

I grew hybrid *P. engelmannii* x *Picea glauca* (Moench) Voss (native interior hybrid spruce), hardened them off, and planted them into the three types of microsites, as described in Chapter 3, Section 3.2.1. I harvested five replicate seedlings from all three microsite types in all three forest plots (5 X 3 X 3 = 45 seedlings with their surrounding substrate) two years after planting, at the end of the growing season in 2009. Each week from August 24th through September 21st, I collected one seedling per microsite at all three plots (1 seedling x 3 microsites x 3 plots = 9 seedlings). For every seedling harvested, I cut a 10 cm wide x 10 cm wide x 20 cm deep block of soil/decayed wood with a pruning saw. The block extended beyond the short lateral roots of these seedlings, and ensure that entire root system was harvested. I gently removed the block using bare hands to ensure that no resistance was felt at the deepest part of the cut that could signal long central roots breaking off. I put the seedling-plus-substrate

sample into a large plastic bag, and kept the bags sealed and on ice until they could be stored at 4 ˚C in the lab.

I processed samples in the lab as soon as was practicable, and always within two days of collection. I gently teased seedlings away from the surrounding soil or decayed wood, and then separated these substrates into bulk and rhizosphere fractions. I considered bulk samples to comprise an approximately 5 cm diameter core surrounding the seedling roots, but that fell away with gentle shaking. I removed all root tips and visible sclerotia from the bulk sample, and ground approximately 20 g with a sterilized mortar and pestle until it was of uniform size and colour. Of this, I used 10 g for dry weight calculations (65 °C for 24 hr), froze 1.0 g at -80 °C in a 1.5 ml microcentrifuge tube for future DNA extraction, and placed 0.1 g into 100 ml autoclaved plastic bottles pre-filled with 50 ml of 5 mM NaAc buffer at pH 5.0 for enzyme assays. I put the samples in assay buffer on a shaker at 200 rpm for one hour. I cleaned the mortar with 70 % ethanol and rinsed with distilled water in between samples.

I considered the soil or decayed wood remaining attached to the roots the rhizosphere fraction, and this was analysed as part of a different study, to be published separately from this thesis, along with additional data on ECM fungi occurring on the roots of the seedlings. In this chapter, I report on the fungal community, abiotic characteristics, and enzyme activities of only the bulk sample fraction.

4.2.3 Microsite abiotic properties

I recorded subsurface moisture and temperature throughout the growing season for all three microsites at all forest plots as described in Chapter 3, Section 3.2.3.

Air-dried and ground subsamples (45 x 10 g) of the bulk fraction were individually tested for mineralizeable N (ammonium, 1 M KCl anaerobic incubation), available nitrate-N and ammonium-N (2 M KCl extraction), available phosphate-P (Bray P-1), and total C and N (combustion elemental analysis). These tests were performed at the Analytical Chemistry Services Lab of the British Columbia Ministry of Forests and Range, Victoria, B.C. I analysed the same soils for pH(H_20) in-house. Loss-on-ignition (Schulte et al., 1996), and the chemistry of the organic carbon were analysed by Lori Ann Phillips (Biology, UBC Okanagan) via proximate analysis into polar extracts comprising simple sugars, proteins and polyphenols (PE), and non-polar extracts comprising fats and waxes (NPE) (Trofymow et al., 1995). The acid soluble fraction (AS), representing cellulose and hemicellulose, and acid insoluble fraction (AIS), representing lignin, cutin, and humics, were also included in the proximate analysis (Trofymow et al., 1995).

4.2.4 Enzyme assays

I assayed substrates in 96-well plates for eight extracellular enzymes: acid phosphatase (EC 3.1.3.2), β-glucosidase (EC 3.2.1.3), β-glucuronidase (EC 3.2.1.31), leucine aminopeptidase (EC 3.4.11.1), β-xylosidase (EC 3.2.1.37), cellobiohydrolase (EC 3.2.1.91), *N*-acetylglucosaminidase (EC 3.2.1.14) and laccase (EC 1.10.3.2). I performed fluorimetric enzyme assays of the hydrolases (acid phosphatase, β-glucosidase, β-glucuronidase, leucine aminopeptidase, β-xylosidase, cellobiohydrolase, and *N*-acetylglucosaminidase), and colourimetric assay of the oxidative enzyme laccase as outlined in Sinsabaugh et al. (2003), with the following modifications: I prepared stock solutions of each substrate (to be used throughout the assay process) only once (the week prior to the entire procedure), except for 4-methylumbelliferone-phosphate, which I made fresh each week for immediate use. I also made working solutions of 10 μM 4-methylumbelliferone and 10 μM 7-amino-4-methyl-coumarin, 50 mM sodium acetate buffer, and 2 mM ABTS (diammonium 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonate, for laccase assays) weekly, and stored them at 4 °C. I removed the soil samples in buffer from the Maax Q 4000 shaker (Barnstead International, Dubuque Iowa) and added an additional 50 ml of buffer prior to pouring the suspension into sterile plastic pipetting troughs. I used a new trough and pipette tips to deliver 200 μl of each soil sample to the microplate wells, and resuspended the soil in the trough by pipetting up and down prior to the delivery of each aliquot. I subsequently augmented this with 50 μl of

substrate per well. I wrapped the microplates in foil to exclude light, and incubated them at 20 °C for the time recommended by Sinsabaugh et al. (2003) for each enzyme. I added a stop solution of 0.5 M NaOH, and read the fluorescence on a FLUOstar Galaxy (BMG Lab Technologies, Ortenburg, Germany) microplate reader at the end of the incubation. I performed laccase assays in clear microplates, and read them in absorbance mode with the excitation filter set at 420 nm. I later converted activities to units of nmol (fluorescence) or μmol (absorbance) of substrate hr^{-1} g^{-1} soil.

4.2.5 Molecular identification of fungi from soil samples

I extracted DNA from previously-frozen, reserved 1.0 g subsamples of the bulk fraction with the MoBio Ultra Clean Soil DNA Extraction Kit using the Alternative Protocol for maximum yields (MoBio Laboratories Inc., Carlsbad CA, U.S.A). Every 50 µl PCR reaction mixture included 5.0 µl 10X buffer, 1.0 µl 10 mM dNTPs, 2.0 µl 50 mM $MgCl_2$, 1.0 µl 10 uM of each forward and reverse fusion primer, 1 U Platinum *Taq* polymerase (Invitrogen Corp, Carlsbad CA, U.S.A.) and 1.0 to 2.0 µl of template DNA (for a final concentration of 0.2 ng ul^{-1}). The sequence of the forward fusion primer was 5'-CCATCTCATCCCTGCGTGTCTCTCCGACTCAG (Titanium A Primer) XXXXXXXXXX CTTGGTCATTTAGAGGAAGTAA (ITS1F)-3' (Gardes and Bruns, 1993), where 'XXX…" represents one of 30 multiplex identifier (MID) tags. The reverse primer for all reactions was 5'-

CCTATCCCCTGTGTGCCTTGGAGTCTCAG (Titanium B Primer) GCTGCGTTCTTCATCGATGC (ITS2)-3' (White et al., 1990). Thermocycler conditions were 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 68 °C for 1 min, and a final extension step of 68 °C for 10 min. I cleaned good quality single bands on 1 % agarose gels with Agencourt Ampure XP magnetic bead PCR purification system (Beckman-Coulter, Danvers MA, U.S.A.), gel-checked them again for band quality and the removal of primer dimers and other low molecular weight product, and quantified them against a low mass DNA ladder and with a NanoDrop micro-volume spectrophotometer (Thermo Scientific, Wilmington, DE. U.S.A.).

In order to tease samples apart for downstream data analysis, I used a unique pyrosequencing primer tag for each substrate sample, and these were physically segregated by plot for sequencing. Consequently, unique molecular information was available for the bulk fraction from every seedling (unlike in Chapter 2). I pooled all amplicons (up to 15 from each plot – 3 microsites x 5 seedlings with their substrate) in an equimolar mixture per plot by combining equal amounts of each PCR amplicon at a standard concentration. The final 20 ng ul^{-1} mixture from each plot, containing up to 15 unique pyrosequencing primers, each representing the bulk soil from one seedling, was amplified in a 1/4 plate Next Generation pyrosequencing reaction on a Roche GS-FLX at the McGill University and Genome Quebec Innovation Center.

4.2.6 Sequence processing and phylogenetic-based naming

I imported sequence and quality files from the pyrosequencing into MOTHUR v. 1.16.0 (Schloss et al., 2009), where I removed primers, and filtered and trimmed sequences (min 100 bp, max 400 bp, pdiffs =1, maxambig = 0, maxhomop = 4). I used the Fungal ITS Extractor (Nilsson et al., 2010) to isolate the ITS1 region, and submitted these sequences to the ITS Pipeline (Nilsson et al., 2009) for matches to the GenBank database (BLASTn, Altschul et al., 1997) with and without uncultured fungi. The ITS Pipeline matches fungal ITS sequences to the GenBank database, and then groups those that share 50 % of their top fifteen closest BLAST database hits, based on their taxonomic names. I aligned the ITS1 sequences using MAFFT v. 5 (Katoh et al., 2002), then assembled into them in distance matrices and clustered them in MOTHUR (countends =F, cutoff=0.10). Specifically, fungal ITS sequences were compared to each other based solely on their DNA alignment because those that match more closely (i.e. their sequences line up) are, in theory, more closely related. The appropriate similarity cutoff for grouping sequences into Operational Taxonomic Units (OTUs) can only be determined, however, by plotting a taxon accumulation curve for a range of similarities (Amend et al., 2010; Buée et al., 2009; Jumpponen and Jones, 2009; Tedersoo et al., 2010b), as explained in Chapter 3, Section 3.2.5. Since I expected the appropriate similarity to be no less than 90 %, I directed MOTHUR to cluster based on 91 % through 99 % sequence similarity. At 91 % molecular similarity, the resulting accumulation curve was flat, suggesting that

the clustered groups were too broad and inclusive, and that the asymptotic curve falsely showed that all taxa had been discovered (which is highly unlikely for these data). Conversely, the curve rose exponentially at 99 % similarity, which suggested that the cluster was too exclusive, and therefore greatly overestimated taxon diversity. I selected a 95 % sequence similarity cutoff curve, which did not reach an asymptote yet did not increase exponentially either, and used this to define the OTUs prior to naming them. It is more common to use 97 % (Buée et al., 2009; Tedersoo et al., 2010b), but 95 % is also used (Jumpponen and Jones, 2009), and the correct choice is often ambiguous (Amend et al., 2010). I imported one randomly chosen representative sequence from each OTU into MEGAN v. 4.40.1 (Huson et al., 2007) for taxonomic placement (based on lowest common ancestor (LCA) parameters: min support = 1, minimum score = 200, top percent = 10, disable = environmental samples). Once the OTUs were grouped taxonomically by MEGAN, I used a conservative approach for subsequent analysis in order to acknowledge short pyrosequencing reads: I grouped and identified most non-ECM OTUs were at the level of order, and named OTUs identified as ECM taxa according to fungal lineage (comparable to family or order, Tedersoo et al., 2010a). I then used the taxonomic information from MEGAN two ways. First, I examined the entire fungal community at a broad taxonomic level (i.e. non-ECM orders and ECM lineages). Second, for better resolution, I analysed the ECM fungal community only using a finer taxonomic level: all OTUs from ECM lineages.

4.2.7 Statistical analyses

The experimental design was a hierarchical model with two factors: plot (random) and microsite (fixed). Substrate abiotic properties, substrate enzyme activity, and fungal community data were fully balanced. For analysis of proximate fractions, temperature, and moisture of the substrate surrounding seedlings, there were 27 samples (3 seedlings x 3 microsites x 3 plots = 27 samples). For all other chemical analyses, enzyme activity, and community analysis, there were 45 samples (5 seedlings x 3 microsites x 3 plots = 45 samples). All of these data are expressed per substrate sample. I tested abiotic properties and enzyme activity data using MANOVA (Statistica v. 6.1; StatSoft Inc., 2003), and tested fungal community data using permutational multivariate ANOVA (Anderson, 2005). I applied multivariate ANOVAs to enzyme data and to community data with the purpose of gauging the response of the entire fungal community. I also tested all response variables with univariate ANOVA (soil properties and enzymes) or permANOVA (fungal community). I applied univariate ANOVAs to enzyme data and community data with the assumption that variables were independent of one another. In all of the above cases I used a mixed-effect hierarchical model, with plot as a random factor and microsite fixed, and with microsite nested in plot. In addition, I used one-way ANOVAs to compare ECM fungal community composition among microsites within individual plots. I transforemd read abundance to presence-absence for analyses of community composition, because read abundance from pyrosequencing is not directly related to

abundance of DNA of a taxon (Amend et al., 2010). I did not eliminate rare OTUs despite a non-normal distribution for most taxa. I log transformed soil enzyme activity and chemical data (with the exception of pH) in order to improve normality and minimize variance. I used post-hoc Bonferroni tests to compare differences between all pairs of means when $p<0.1$ in the ANOVA, and I applied a Bonferroni correction when considering results of univariate tests of soil properties. Examples of all the models can be found in the appendix (Tables S4.1-S4.7), and they are referred to in the relevant section in the text.

I used Nonmetric Multidimensional Scaling (NMS) in PCORD v. 5.0 (McCune and Mefford, 1999) to visually explore the relative relationships among fungal communities for the reasons explained in Chapter 3, Section 3.2.6. I also explored this dataset with Principal Components Analysis (PCA), which documents covariance among linear variables such as those generated by the transformation of environmental data (Kenkel, 2006; Borcard et al., 2011). The ordination of heterogeneous community data that is characterized by non-linear species response to underlying variables is not amenable to PCA (McCune and Grace, 2002; Kenkel, 2006) without data pre-transformation (Kenkel, 2006, Borcard et al., 2011); however, my analyses of fungal taxon presence-absence was an acceptable transformation, although it required the deletion of all taxa that were uniformly present in all samples (i.e. those with no variance on a 0,1 scale). This meant that dominant taxa, which were not important for structuring the ordination because they were shared by all samples, were not included in the

ordination. Joint biplots identifying important soil chemical properties and enzyme activity were constructed with a second matrix containing these variables.

4.3 Results

4.3.1 Soil abiotic properties

The three types of microsites varied considerably in their chemistry, with decayed wood generally differing from mineral soil. Not surprisingly, total C and % organic matter were highest in decayed wood, and pH was lowest in decayed wood (Table 4.1, 4.2, and C.1). Of the components of organic matter, only polar extractables (representing simple sugars, proteins and polyphenols) and non-polar extractables (fats and waxes) differed among microsites (Table 4.2). Of the mineral nutrients, available P differed weakly among microsites (Table 4.1). The highest levels of these three properties were found in decayed wood and the lowest in control soils. Plot effects were detected for some soil nutrients, and some organic components, as well as for pH; plot A had significantly lower pH, lower levels of nitrate-N, and higher non-polar extractables than one or both of the other two plots (data not shown).

Table 4.1 Soil chemical properties for microsites in mature forest plots. Values represent means (SD) based on untransformed data. Significant differences per column at p ≤ 0.1 are in bold type and are based on log-transformed data analysed with a univariate hierarchical ANOVA. N=5 per microsite per each of three plots. MANOVA p<0.0001.

Microsite	pH (H_2O)	%		mg kg^{-1}			
		Total C	Total N	Available P	Available NH4-N	Available NO3-N	Mineralizeable NH4-N
Control soil	4.9 (0.57)	17.8 (11.6)	0.78 (0.44)	11.8 (9.1)	24.5 (20.0)	29.1 (29.1)	352.9 (173.2)
Downed wood	4.7 (0.51)	26.0 (16.0)	1.02 (0.54)	16.3 (10.0)	41.1 (33.0)	20.6 (29.0)	462.2 (209.8)
Decayed wood	4.4 (0.41)	54.3 (7.8)	0.71 (0.17)	18.3 (7.4)	21.2 (20.9)	17.2 (9.5)	272.0 (92.6)
p	**0.005**	**<0.0001**	0.45	**0.04**	0.39	0.15	0.12

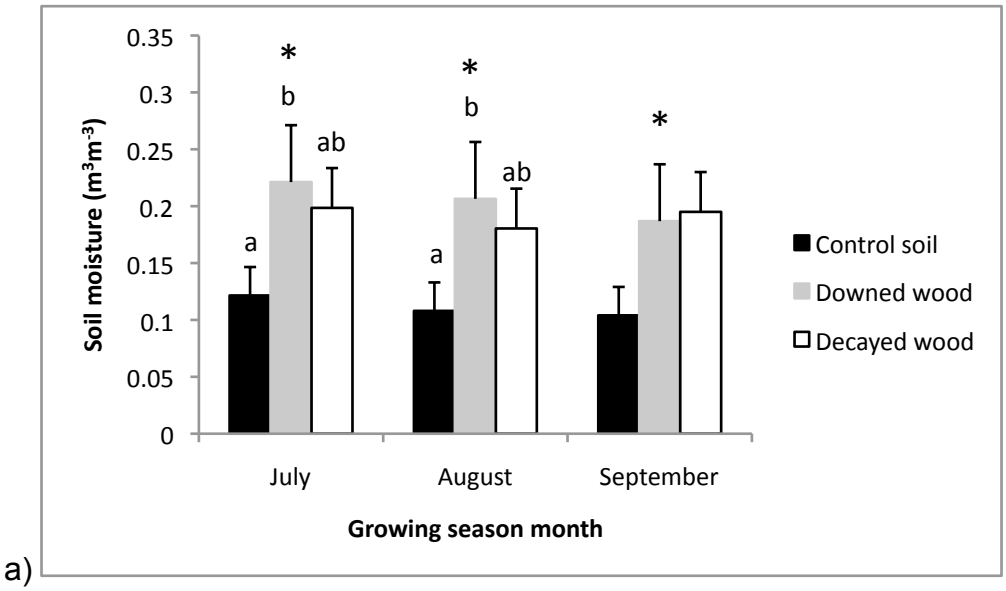
Table 4.2 Soil carbon fraction for microsites in three forest plots. Values represent means (SD) based on untransformed data. Significant differences per column at p ≤ 0.1 are in bold type and are based on log-transformed data analysed with a univariate hierarchical ANOVA. N=3 per microsite per each of three plots. MANOVA p=0.0001.

Microsite	% Organic matter	mg g^{-1}			
		Non-polar extractables	Polar extractables	Acid soluble	Acid insoluble
Control soil	28.0 (10.9)	11.6 (9.8)	25.9 (13.0)	215.1 (91.4)	730.0 (121.5)
Downed wood	42.3 (24.1)	18.0 (14.0)	36.9 (25.1)	171.1 (58.0)	791.4 (67.5)
Decayed wood	96.0 (2.6)	20.3 (8.2)	73.8 (15.7)	261.3 (42.8)	644.6 (57.9)
p	**0.0002**	**0.08**	**0.001**	0.22	0.12

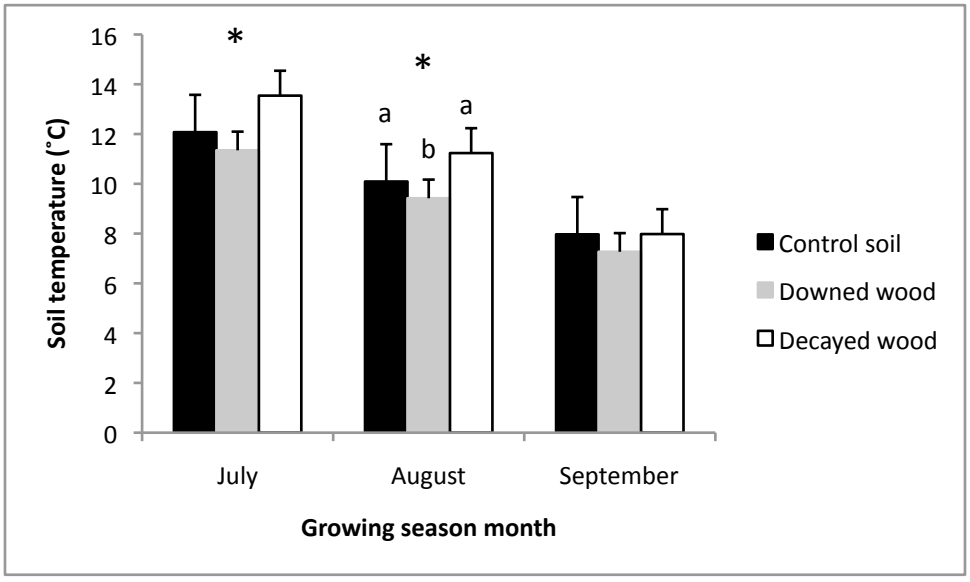
Soil moisture and soil temperature differed among the three microsites throughout the growing season. There was a difference in minimum daily soil moisture among microsites when data for all three summers (2007, 2008, and 2009) were compared per month (July $p=0.0001$, August $p<0.0001$, and September $p=0.0001$). In general, soil moisture was lower in control soil than in the downed wood microsites (Figure 4.1a; Table C.2a), with significant plot effects; soil moisture was lowest in plot A in each growing season month ($p\leq0.002$, data not shown). Soil temperature tended to be lowest in downed wood microsites, and this difference was significant in July ($p=0.054$) and August ($p<0.0001$) over all three years (Fig 4.1b; Table C.2b). Soil temperature differed among plots only in September ($p=0.066$); temperature was highest in plot B.

4.3.2 Soil enzyme activity

Enzyme activities varied little among microsites. The overall fungal community soil enzyme profile (interpreted by testing all eight enzymes with a multivariate hierarchical ANOVA) did not differ among microsites (Table 4.3). Xylosidase was the only enzyme whose activity differed by microsite when tested independently. Xylosidase activity was lowest in decayed wood, however this was only weakly significant when considering the presence of other enzyme variables (Table 4.4). Plot effects were significant for five of the eight enzymes, and all activities but laccase were highest at plot A (data not shown).



a)



b)

Figure 4.1 a) Mean minimum daily soil moisture over each growing season month among microsites, and b) mean maximum daily soil temperature over each growing season month among microsites.
Daily soil moisture and temperature were averaged per month, and the data from each month was compared over three study years. Error bars denote mean values +SEM, and asterisks denote overall significant differences among microsites at $p \leq 0.1$. Letters above column signify differences among means at $p \leq 0.1$ based on post-hoc Bonferroni tests. N=3 per microsite (one per plot) per year.

Table 4.3 Multivariate hierarchical ANOVA of the community enzyme profile (i.e. all eight enzymes) among microsites and plots.

	Test	Value	F	Effect	Error	p
Intercept	Wilks	0.003	1590.1	7	30	0.000
Plot	Wilks	0.012	35.3	14	60	**0.000**
Microsite(Plot)	Wilks	0.241	1.2	42	144.2	0.198

Table 4.4 Fungal community enzyme activity per microsite. Values represent means (SD) of untransformed data; p-values are based on hierarchical ANOVA of log-transformed data. N=5.

	pmol mm^{-2} min^{-1}			
Microsite	Glucuronidase	Xylosidase	Cellobiohydrolase	Glucosidase
Control soil	966.2 (2349.4)	188.7 (206.2)	301.9 (387.5)	982.1 (810.0)
Downed wood	481.7 (1831.0)	176.3 (208.2)	416.7 (366.8)	1417.2 (1113.7)
Decayed wood	33.1 (33.8)	72.7 (80.5)	341.6 (221.0)	1228.8 (878.9)
p	0.369	0.061	0.703	0.637

	pmol mm^{-2} min^{-1}			mmol mm^{-2} min^{-1}
Microsite	Chitinase	Aminopeptidase	Phosphatase	Laccase
Control soil	433.4 (313.9)	58.1 (81.6)	2891.4 (1878.9)	12.5 (8.32)
Downed wood	830.5 (867.9)	95.5 (90.6)	3832.5 (2003.3)	11.3 (9.19)
Decayed wood	621.5 (673.2)	48.5 (81.3)	3101.2 (1810.4)	13.3(24.3)
p	0.185	0.728	0.242	0.275

4.3.3 Abundance and identification of fungal operational taxonomic units (OTUs)

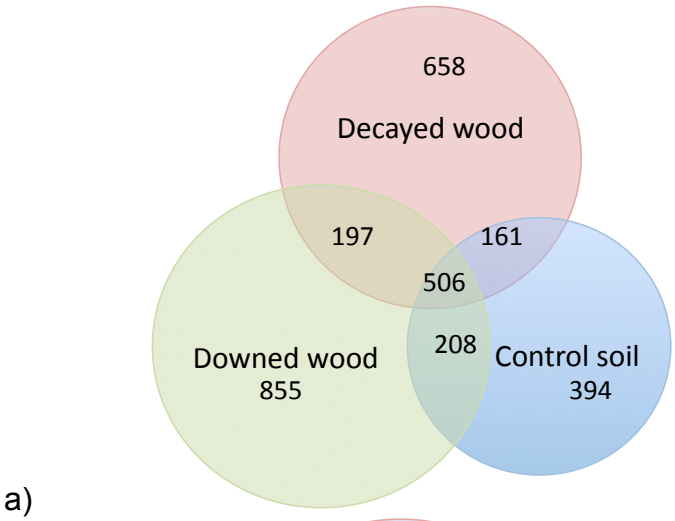
The total number of pyrosequencing reads and the subsequent number of taxonomic groups (OTUs) differed among plots, and among microsites within plots. Following all sequence editing, the number of pyrosequencing reads and the number of unique sequences per plot were much lower at plot A than they were at plots B and C (Table 4.5), in spite of identical initial amounts of DNA. The number of fungal OTUs per plot (based on 95 % sequence similarity) were also lowest at plot A. For all plots, over 50 % of the OTUs were singletons and

doubletons (Table 4.5), and, in general, there were more OTUs unique to one microsite in a plot than there were OTUs shared among all microsites within each plot (Table 4.5; Figure 4.2). While there were clear differences among microsites at each plot when all OTUs were considered (Figure 4.2), rarefaction curves for the five samples per microsite per plot suggested that the plots differed even more from each other (Figure 4.3). Additionally, none of these curves were asymptotic suggesting that many fungal taxa remain undetected in spite of the deep sequencing effort.

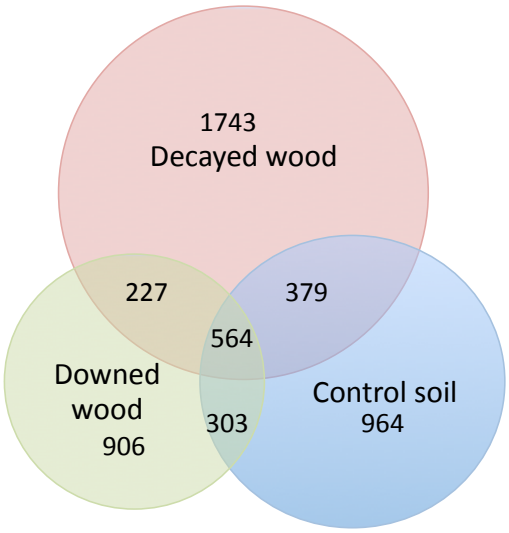
Table 4.5 Summary of processing of pyrosequencing data showing the number and proportion of reads, sequences, and OTUs among plots and microsites in a mature spruce-fir forest.

	Plot A	**Plot B**	**Plot C**
Number of original reads[1]	122616	197568	174984
Final edited ITS reads[2]	89908	169327	147232
Unique sequences within edited reads[3]	20883	39125	43464
Mean number of edited reads per sample (SD)[4]	5994 (3578)	11288 (3753)	10517 (4888)
Number of operational taxonomic units (OTUs)	2979	5086	5804
Number of singleton and doubleton OTUs[5]	1560	2656	2992
Number of OTUs in control soil	1269	2210	2663
Number of OTUs in downed wood	1766	2000	2401
Number of OTUs in decayed wood	1522	2913	2780
Number of OTUs shared by all microsites	506	564	444

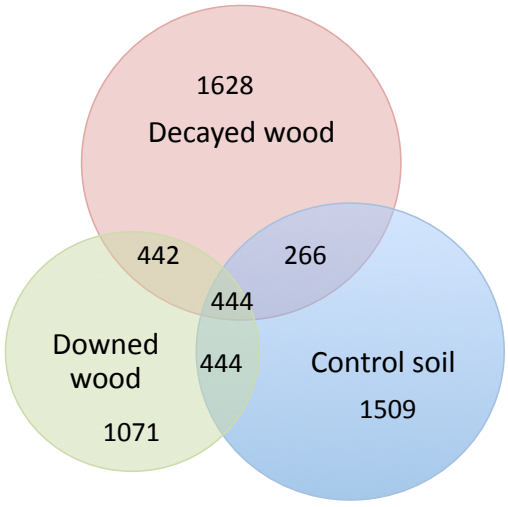
[1] Raw pyrosequencing data prior to any sequence editing.
[2] Filtered and trimmed sequences after all quality control (e.g. minimum 100 bp).
[3] This is comparable to grouping the OTUs at 100% similarity.
[4] Number of reads generated from each individual seedling substrate sample.
[5] OTUs containing zero or two reads (i.e. potential artifacts).



a)



b)



c)

Figure 4.2 Venn diagram of all shared and unique OTUs (including singletons and doubletons) in a) forest plot A, b) forest plot B, and c) forest plot C microsites. OTUs are shared among microsites where adjacent circles overlap. Areas of overlap and size of circles are not to scale.

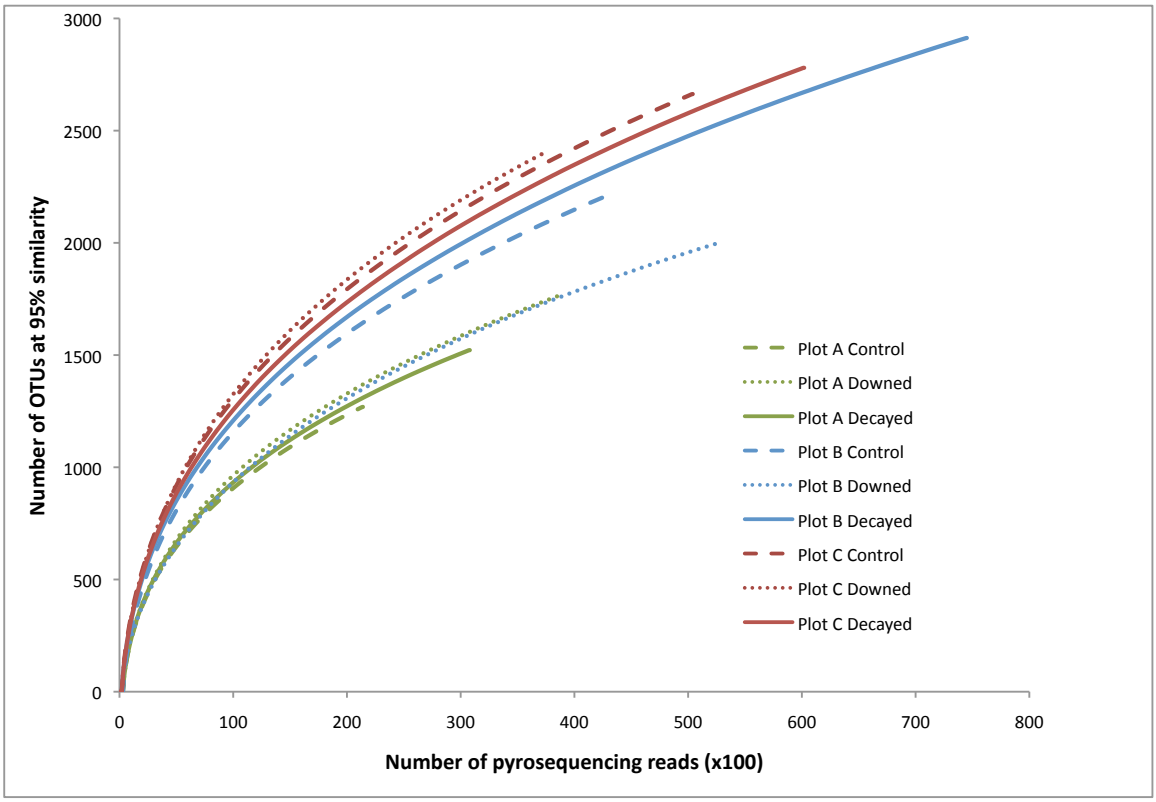


Figure 4.3 Rarefaction curves showing the number of fungal OTUs detected in each microsite at every plot at 95% molecular similarity.

I used OTUs with at least 100 reads for all subsequent analyses (Table 4.6). This was in order to manage the very large dataset, and it ensured that OTUs reflected real taxonomic groups, and not simply pyrosequencing artifacts. Analyses of OTUs with at least 100 reads refers to those performed on 37 taxonomic groups at the level of family or higher (including 17 ECM lineages and 20 other fungal orders, classes, or phyla) and to the analyses of 99 ECM OTUs (each of which represented a different ECM species). Although fewer than 3 % of OTUs contained more than 100 sequences, these represented approximately 75 % of the total reads per plot (Table 4.6).

Table 4.6 The number of OTUs at each plot containing at least 100 pyrosequencing reads, and the proportion of reads these represent.

Bulk forest substrate summary	Plot A	Plot B	Plot C
Number of OTUs with at least 100 reads[1]	78	135	145
% of OTUs represented	2.6%	2.7%	2.5%
Number of reads represented	66958	129911	101280
% of reads represented	74.50%	76.70%	68.60%

[1]A few of these OTUs are shared among plots; the total number of unique OTUs containing 100 reads is 267 (99 of which are ECM fungal taxa).

Across all samples, the most abundant higher-level taxonomic group of all fungi (based on the number of reads, and compared to all other OTUs with greater than 100 sequences) was the ECM lineage /amphinema-tylospora (Table 4.7). DNA of the ECM lineage /hygrophorus and the saprotrophic order Mortierellales also amplified frequently from these soils, but distribution of these fungal groups appeared to differ among plots (Table 4.7). In addition to those shown in Table 4.7, the ECM lineages /wilcoxina (0.8 %), /meliniomyces (0.6 %), and /tomentella-thelephora (0.5 %), were among the groups containing OTUs with greater than 100 reads, but that made up only a small proportion of the community overall (data not shown). The differences among plots suggested by the distribution of higher taxonomic groups (above) were supported by a finer-scale view of the fungal community provided by a Venn diagram of the 267 fungal OTUs containing at least 100 reads (Figure 4.4).

Table 4.7 The most abundant ECM lineages per plot (based on read abundance of all identified OTUs with greater than 100 reads) compared to their overall abundance and to the abundance of the saprotrophic order Mortierellales.

ECM lineage or fungal family[1]	Plot A	Plot B	Plot C	Overall
/amphinema-tylospora	20.0%	23.8%	17.2%	20.7%
/hygrophorus	31.5%	9.7%	2.4%	12.1%
/piloderma	13.6%	12.0%	0.3%	8.4%
/russula-lactarius	3.1%	5.3%	15.4%	8.2%
/pseudotomentella	1.8%	5.6%	1.7%	3.4%
/inocybe	1.3%	2.1%	3.2%	2.3%
/cortinarius	5.3%	1.0%	1.5%	2.1%
Mortierellales	3.8%	12.3%	15.1%	11.3%
Proportion of soil community[2]	**80.4%**	**71.8%**	**56.8%**	**68.5%**

[1]These eight fungal taxa represent the top five higher-level taxa from each plot.
[2]Total contribution of these eight taxa to the entire fungal community detected in forest soils (i.e. including OTUs with fewer than 100 reads).

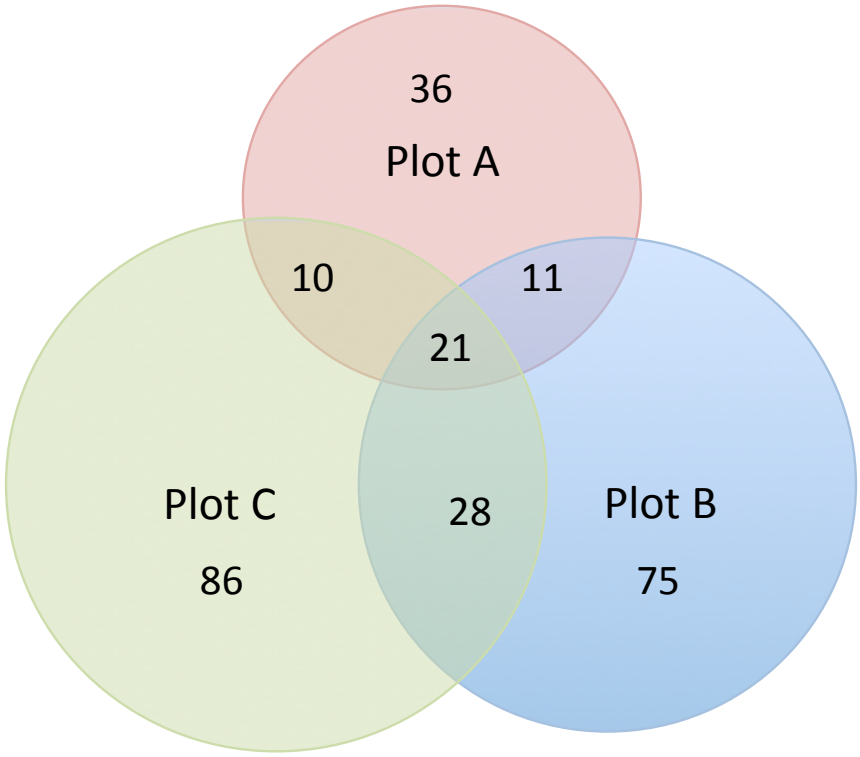


Figure 4.4 Venn diagram of the distribution of 267 unique OTUs among forest plots A, B, and C. OTUs are shared among plots where circles overlap, but areas of overlap and size of circles are not to scale. These OTUs include 99 ECM taxa, 18 members of the Mortierellales, and 60 unknowns, among other fungal groups (see Table C.3a-c for the number of taxa within a fungal group).

4.3.4 Diversity and composition of fungal communities

I detected differences in the taxonomic composition of fungal communities among plots, but not among microsites, when the communities were examined using higher-level taxa of all fungi (Table 4.8). Ordinations illustrated that samples clustered primarily by plot (Figure 4.5), especially along axis 3 of the NMS ordination (Figure 4.5a) and axis 1 of the PCA (Figure 4.5b). However many taxa were shared between plots B and C, as can be seen by the clumped nature of the taxa near the middle of the plot. Collectively, community composition did not differ among microsites (Table 4.8); however, independent univariate ANOVAs of higher-level taxon presence-absence revealed that the mitosporic Ascomycota (p=0.042), and unknown Basidiomycota (p=0.013) differed weakly among microsites. ECM lineages differed only by plot (Table C.4; C.5; C.6).

Table 4.8 Multivariate permutational ANOVA of per sample presence-absence for the entire community of all higher level fungal taxa, including ECM lineages, among control soil, downed, and decayed wood microsites and among plots.

	Degrees of freedom	SS	MS	F	P(perm)
Plot	2	8699.1	4349.5	15.5	**0.001**
Microsite(Plot)	6	2129.5	354.9	1.27	0.193
Residual	36	10079.1	280.0		
Total	44	20907.7			

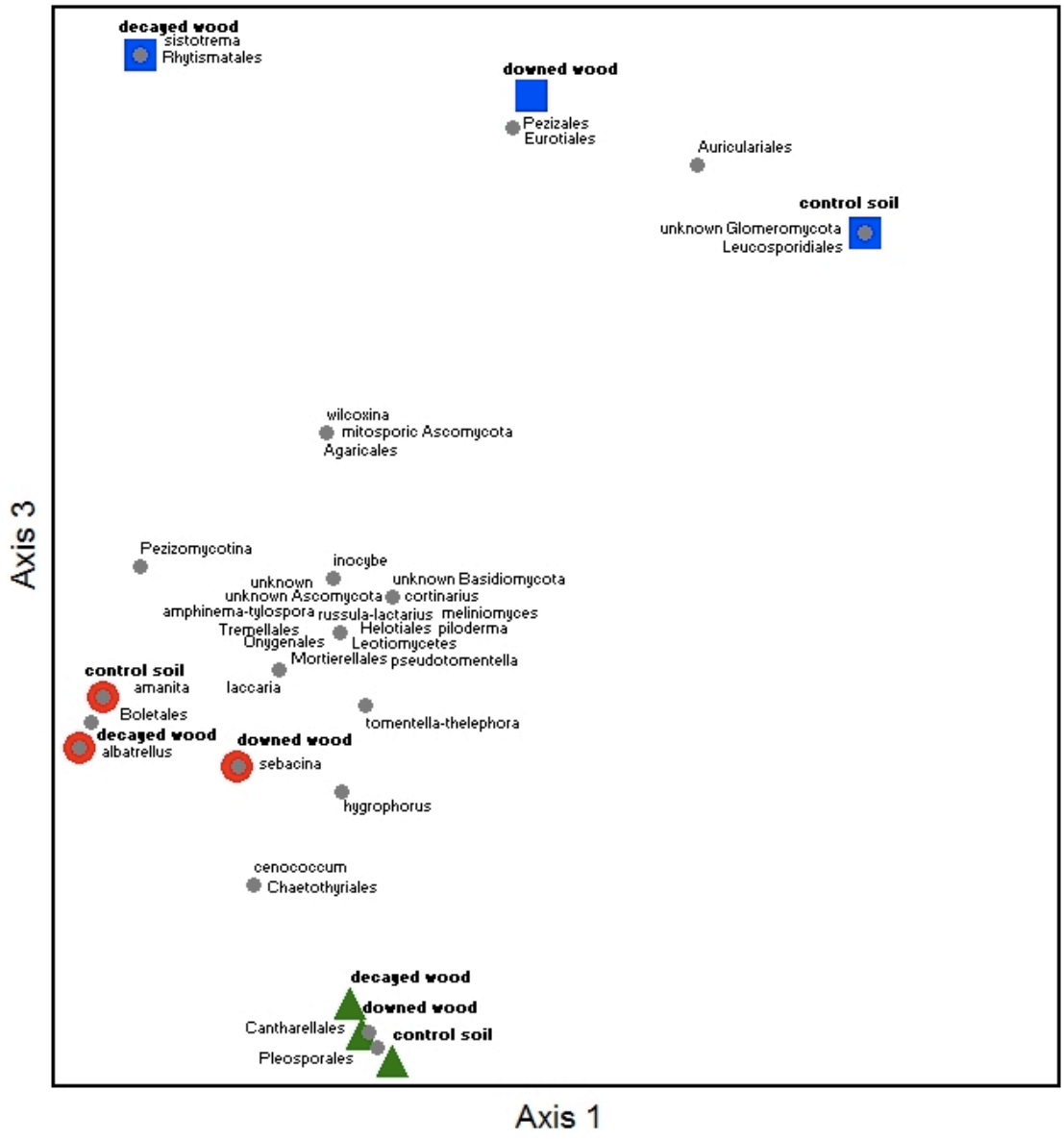


Figure 4.5a NMS ordination of higher-level fungal taxa (ECM lineages, and orders or higher taxa of other fungal groups) per microsite per plot.
Stress is excellent (0.64), and the total variation explained = 95.9% (axis 1 = 0.194, axis 2 = 0.091, and axis 3 = 0.746). Green triangles represent plot A, red circles plot B, and blue squares plot C.

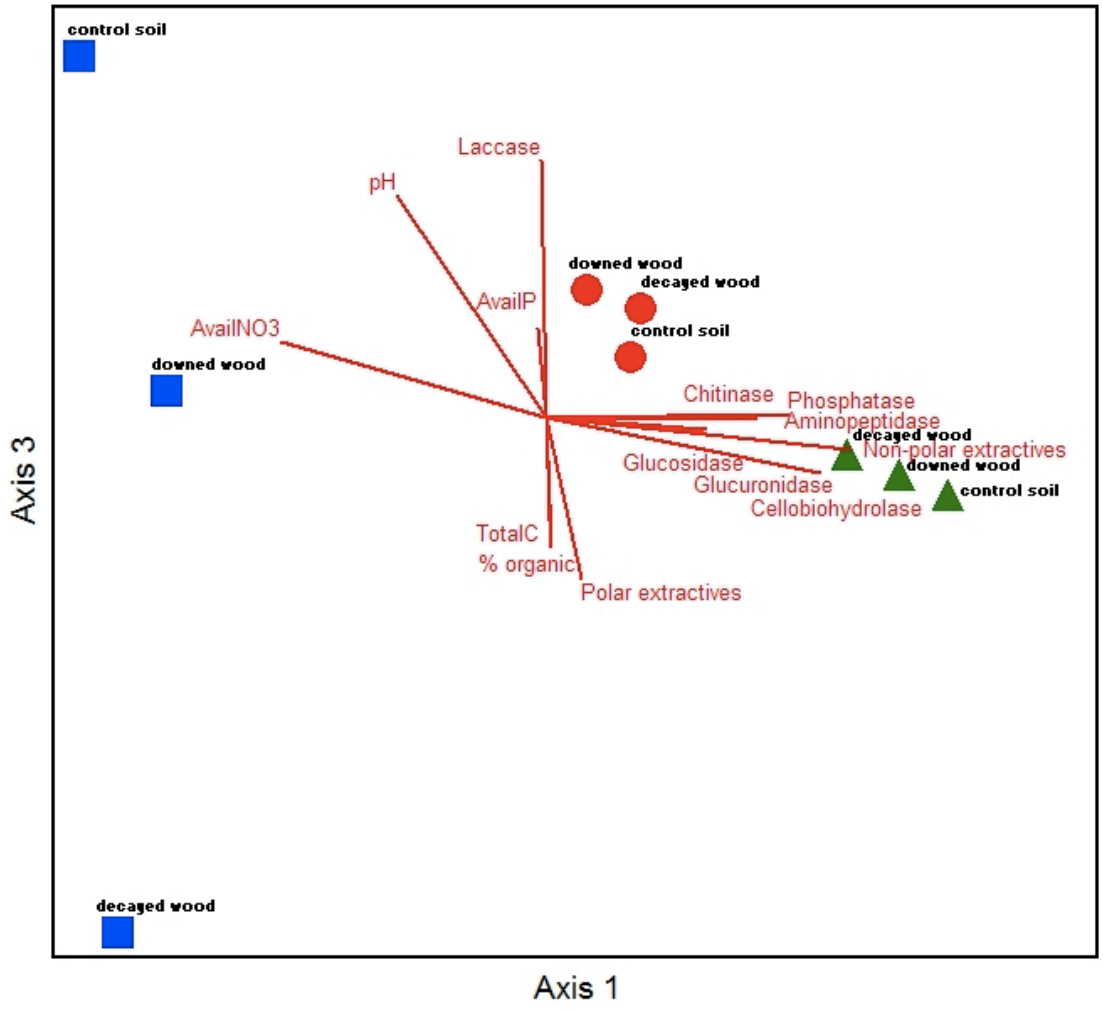


Figure 4.5b PCA biplot of higher-level fungal taxa (ECM lineages, and orders or higher taxa of other fungal groups) per microsite per plot.
The total variation explained = 91.6% (axis 1 = 0.723, axis 2 = 0.148, and axis 3 = 0.046); the inflation factor is 9.02. Green triangles represent plot A, red circles plot B, and blue squares plot C.

I compared assemblages of the 99 ECM fungal OTUs with greater than 100 reads in order to examine microsite effects on ECM fungi in more depth (i.e. in order to analyse the ECM fungal community at the level of species). Collectively, the composition of this group differed only by plot (Table 4.9). However, the occurrence of a number of ECM species differed significantly at $p \leq 0.10$ among microsites (Table 4.10; Table C.8a) and plots (data not shown) when analysed independently. Post-hoc Bonferroni tests showed that for many of these species, the significant microsite differences occurred at only one of the three plots. Given this, and the general conclusion that soil fungal communities varied considerably among the plots, I analysed the assemblage of ECM species more thoroughly within each plot. I detected differences among microsites for the entire ECM community only at Plot C ($p=0.058$; Table C.7; Figure 4.6c). However, based on independent univariate ANOVAs, some ECM species were more likely to be present in certain microsites in specific plots (Table 4.10; Table C.8b). For example, in Plot A, /amphinema-tylospora4 occurred less frequently in downed wood microsites (Figure 4.6a; Table C.8b). Similarly, /piloderma10 (likely *P. croceum*) was absent from decayed wood microsites in plot B (Table C.8b) and is appropriately found directly opposite from all decayed wood icons in the ordination (Figure 4.6b). In plot C (Figure 4.6c), a number of ECM taxa can be found associated with microsite icons with which they are aligned, including /meliniomyces5 (*C. finlandica*), which was most frequent in decayed wood, and /amphinema-tylospora13 (*A. byssoides*), which was frequent in mineral soil and decayed wood microsites (Table C.8b). Contrasting patterns can be seen for

/amphinema-tylospora6 (*T. fibrillosa*) and /amphinema-tylospora20 (*T. asterophora*): the former is frequent in downed and decayed wood microsites, while the former most frequent in control soil (Table C.8b). The differences detected among microsites within individual plots is reflected in overall patterns across plots for all taxa except /amphinema-tylospora4 (Table 4.10; Table C.8a).

Table 4.9 Multivariate permutational ANOVA of per sample presence-absence, for the community assemblage of all ectomycorrhizal species among plots and among microsites within plots. N=5 samples per microsite per plot.

	Degrees of freedom	SS	MS	F	Permutational p-value
Plot	2	39877.2	19938.6	16.1	**0.001**
Microsite(Plot)	6	9222.5	1537.1	1.24	0.155
Residual	36	44502.5	1236.2		
Total	44	92603.3			

Table 4.10 ECM fungal species whose occurrence varied among microsites overall, and/or among microsites within a single plot, when tested independently with one-way permutational ANOVAs.

Plot	Taxon name[1]	Likely identity[2]	Overall p	Plot p
A	/amphinema-tylospora4	*Tylospora fibrillosa*	1.000	**0.086**
	/cortinarius2	*Cortinarius caperatus*	**0.022**	0.194
B	/meliniomyces1	*Meliniomyces bicolor*	**0.067**	0.190
	/piloderma10	*Piloderma croceum*	**0.001**	**0.080**
C	/amphinema-tylospora6	*Tylospora fibrillosa*	**0.045**	**0.081**
	/amphinema-tylospora13	*Amphinema byssoides*	**0.001**	**0.007**
	/amphinema-tylospora20	*Tylospora asterophora*	**0.001**	**0.087**
	/laccaria3	*Laccaria nobilis*	**0.035**	0.251
	/meliniomyces5	*Cadophora finlandica*	**0.001**	**0.052**
	/pseudotomentella7	*Pseudotomentella tristis*	**0.084**	0.306

[1]Taxon names are derived from the lineage within which the taxon belongs and the number of different taxa within the same group.
[2]Identities are based on the best BLAST and UNITE database hits for each taxon; these can be found in Table C.3a-c.

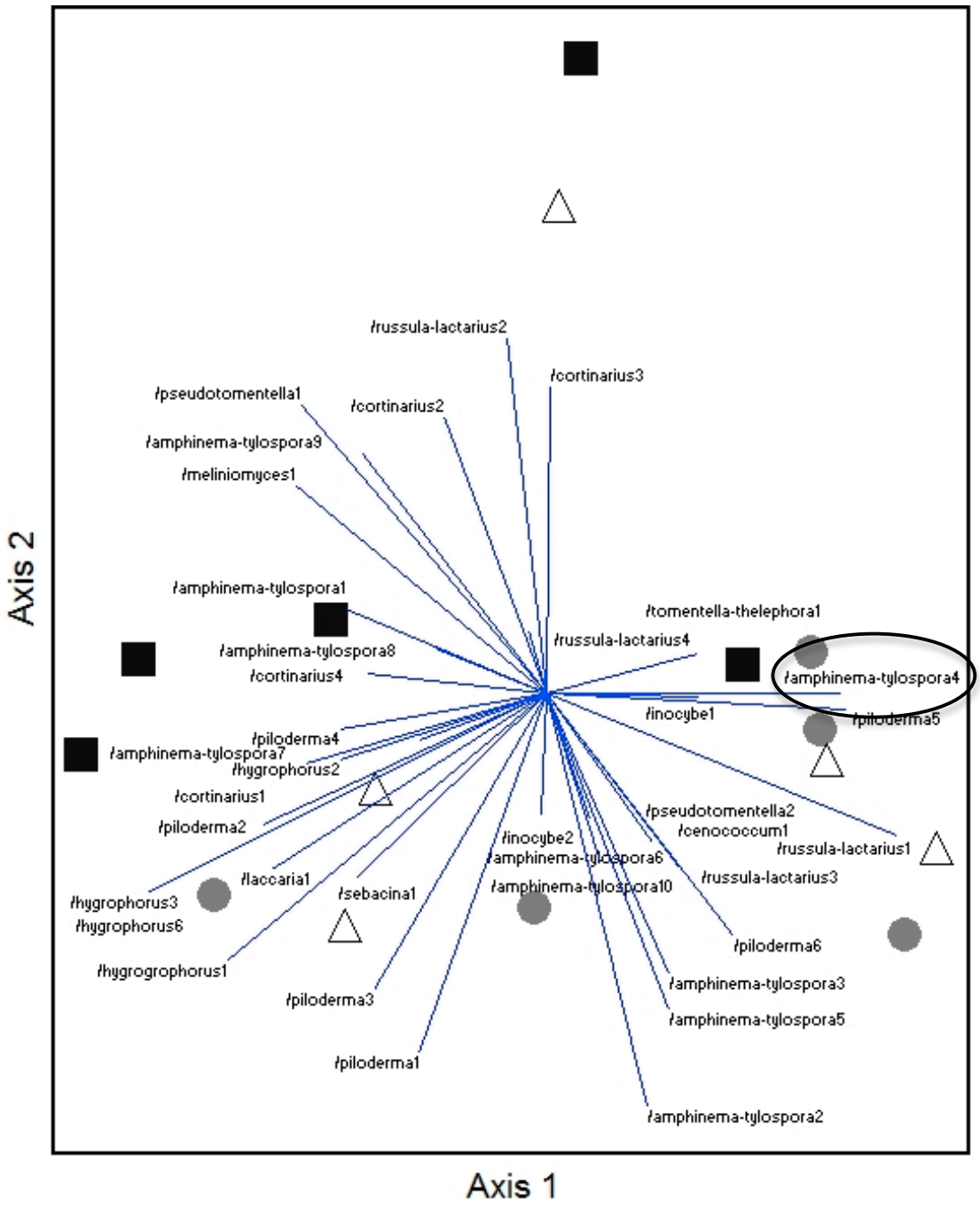


Figure 4.6a PCA ordination of ECM taxon occurrence among microsites at plot A.
The total variation explained by all axes is: A = 76.7% (axis 1 = 0.325, axis 2 = 0.215, and axis 3 = 0.227); B = 66.6% (axis 1 = 0.244, axis 2 = 0.164, and axis 3 = 0.259); C = 62.1 % (axis 1 = 0.346, axis 2 = 0.093, and axis 3 = 0.182). Grey circles represent decayed wood, black squares downed wood, and open triangles control soil. Taxon labels follow the conventions described for lineages, and the closest matching fungal species can be found in Table C.3a-c. Circles indicate taxa referred to in the text. N=5.

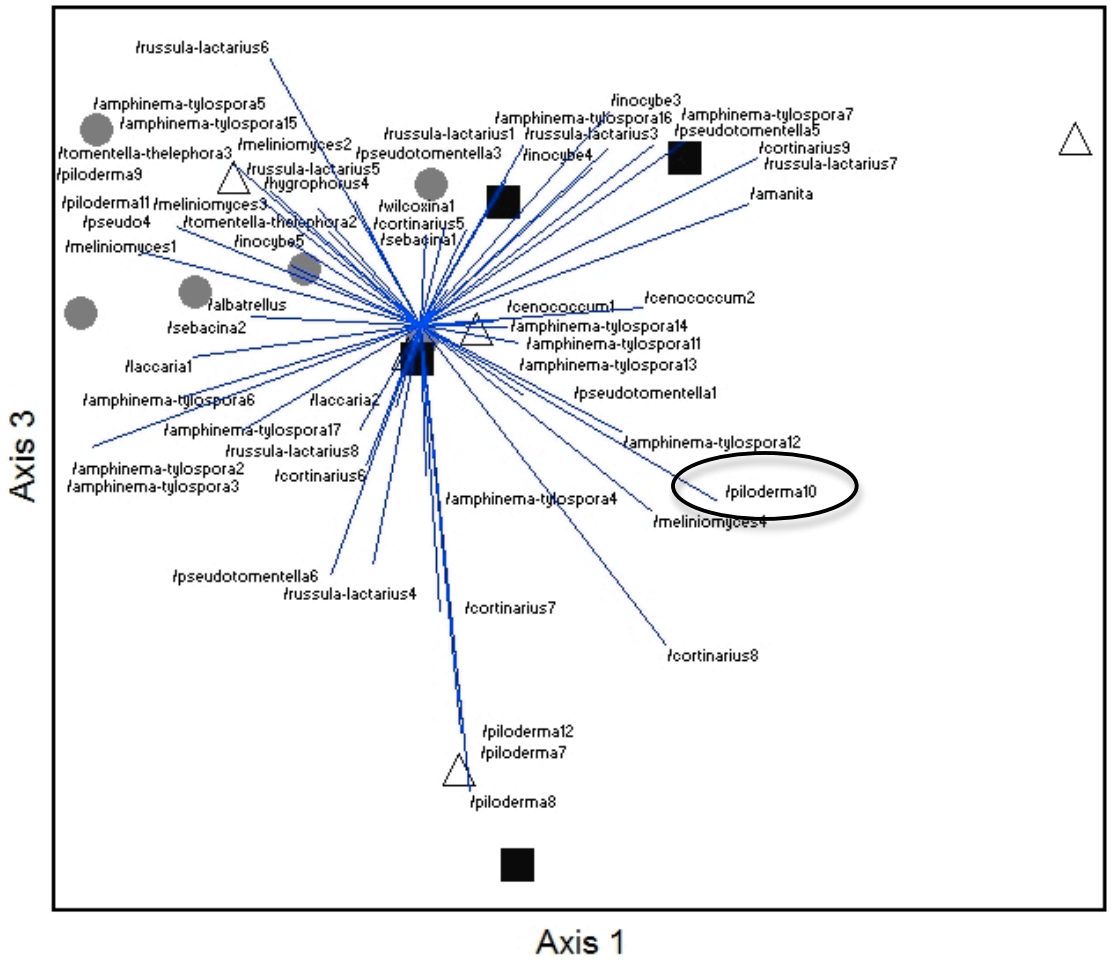


Figure 4.6b PCA ordination of ECM taxon occurrence among microsites at plot B.
The total variation explained by all axes is: A = 76.7% (axis 1 = 0.325, axis 2 = 0.215, and axis 3 = 0.227); B = 66.6% (axis 1 = 0.244, axis 2 = 0.164, and axis 3 = 0.259); C = 62.1 % (axis 1 = 0.346, axis 2 = 0.093, and axis 3 = 0.182). Grey circles represent decayed wood, black squares downed wood, and open triangles control soil. Taxon labels follow the conventions described for lineages, and the closest matching fungal species can be found in Table C.3a-c. Circles indicate taxa referred to in the text. N=5.

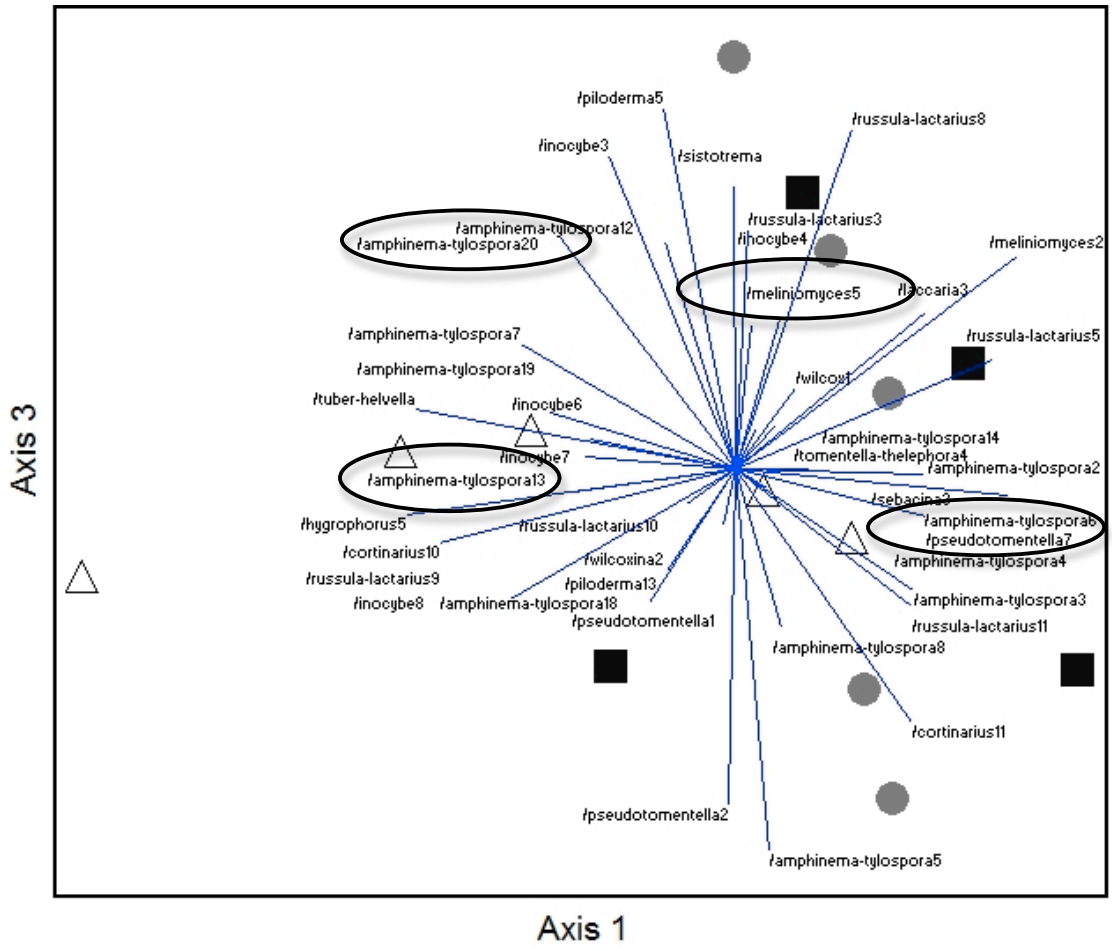


Figure 4.6c PCA ordinations of ECM taxon occurrence among microsites at plot C.
The total variation explained by all axes is: A = 76.7% (axis 1 = 0.325, axis 2 = 0.215, and axis 3 = 0.227); B = 66.6% (axis 1 = 0.244, axis 2 = 0.164, and axis 3 = 0.259); C = 62.1 % (axis 1 = 0.346, axis 2 = 0.093, and axis 3 = 0.182). Grey circles represent decayed wood, black squares downed wood, and open triangles control soil. Taxon labels follow the conventions described for lineages, and the closest matching fungal species can be found in Table C.3a-c. Circles indicate taxa referred to in the text. N=5.

4.3.5 Relationship among enzyme profiles, abiotic factors, and fungal communities

Community enzyme profiles and soil abiotic properties varied primarily at the plot scale, and a PCA ordination of the distribution of higher-level taxa of all fungi, including ECM lineages (Figure 4.5b), best illustrates how some of these properties were aligned with the fungal community in different forest plots. The most important continuous variables are represented by vectors of increasing length, emanating from the center of the ordination. Variables that did not contribute to the equation defining this ordination (e.g. xylosidase and Total N) were not plotted, since they had no meaningful position on the axes shown (McCune and Grace, 2002). The variation among fungal communities in Figure 4.5b is best explained by axis 1, and the vectors for six of the eight enzymes run roughly parallel to this axis, indicating that activities of these enzymes were highest in plot A and lowest in plot C. Plot A had a unique fungal community when assessed by the distribution of higher level taxa. Specifically, it was dominated by the ECM lineage /hygrophorus, which was proportionally more abundant at plot A than at the other plots (Table 4.7). The affinity of /hygrophorus to plot A is not obvious in Figure 4.5a because OTUs from the /hygrophorus lineage were present in all plots. The fungal orders Cantharellales and Pleosporales are unique to plot A (Figure 4.5a; Table C.3). By contrast, the vectors of most of the soil chemistry variables were oriented perpendicularly to the enzyme vectors, with the exceptions of nitrate and non-polar extractives. In general, nitrate was lower and non-polar extractives were higher at plots where

enzyme activities were higher. Soil pH is also lower where enzyme activities are higher, although this is not strongly reflected in the perspective given by these axes (i.e. pH is not aligned with laccase when viewed with axis 2). The vector for laccase is parallel to axis 3, which is the same axis along which the three microsites of plot C separate. Laccase appeared to be higher in samples with lower total C, % organic matter and polar extractives, however this axis explains little of the variation.

4.4 Discussion

4.4.1 Fungal community composition differed less than expected among microsites

I observed that the composition of all fungal OTUs differed among microsites within each plot, with very few OTUs shared among microsites of decayed wood, downed wood, and mineral soil. However, I detected no differences among microsites for the overall fungal community at a coarse taxonomic level, nor for the finer level of ECM species, when only the dominant OTUs were considered. Interestingly, while there was no shift in the entire ECM community, the occurrence of some ECM fungal species varied by microsite. For example, *Tylospora* spp., *Amphinema byssoides*, *Piloderma croceum*, and *Cadophora finlandica* were consistently found in, or were absent from certain microsites. The presence of other individual taxa also appeared to demonstrate a pattern related to woody microsites. Nevertheless, the unique responses of these individual taxa

were not reflected in the overall community pattern, which is not uncommon in studies of fungal community structure (Taylor et al. 2010).

In this study, *Tylospora fibrillosa* and *A. byssoides* (both members of the ECM order Atheliales) were consistently present in decayed wood microsites; *T. asterophora* was more frequent in mineral soils. Tedersoo et al. (2008) found *T. fibrillosa* and *A. byssoides* to be the dominant ECM taxa on rotted logs in an undisturbed spruce forest in Estonia; these taxa were also relatively frequent in most forest microsites, including the undisturbed forest floor. Little competitive exclusion has previously been observed between *A. byssoides* and *T. fibrillosa*, since they have different growth forms (Agerer, 2001; Tedersoo et al., 2008). It is not surprising that these species co-occur in this study. Landeweert et al. (2003) found that the mycelia of *T. asterophora* were only detected in the strongly weathered E horizon of a spruce and pine forest in northern Sweden, a soil layer where total C was low. This is consistent with my detection of *T. asterophora* in nutrient poor control soil. Ecological and physiological differences are common among fungal genera and among fungal species (Kranabetter et al., 2009; Lilleskov et al., 2011; Smith and Read, 2008). Therefore, while these three species occur in similar habitats in the Northern Hemisphere, it is not surprising that the two species within the Genus *Tylospora* are adapted to two different substrates at Sicamous Creek.

Piloderma spp. are often considered as taxa that favour decayed wood microsites for mycorrhiza development, and some species are only abundant in older forests (Twieg et al., 2007; Smith et al., 2000). For example, *P. fallax* mycorrhizae appear to consistently prefer, and in fact require, decayed wood substrates (Goodman and Trofymow, 1998; Smith et al., 2000). While the root tips (Rosling et al., 2003) and hyphae (Landeweert et al., 2003) of *Piloderma* OTUs have been found in all soil horizons, the mycelia of *Piloderma* were more abundant in C-enriched (Landeweert et al*.,* 2003) and mineral N-poor (Lilleskov et al., 2002) spruce forest soils. Therefore, the absence of *Piloderma croceum* (/piloderma10) from decayed wood in my study was unexpected. This may have been related to the identity of plant host roots in the decayed wood. For example, *P. croceum* mycorrhizae have been found on the roots of western hemlock seedlings (Christy et al., 1982)*,* but not on the roots of pine seedlings (Iwanski and Rudawska, 2007) growing in decayed wood. However, *Piloderma* spp. are known to colonize the soils surrounding both spruce (Arocena et al., 2001) and fir (Arocena et al., 1999), which are the dominant host trees at Sicamous Creek. I cannot explain the absence of this *Piloderma* species from the decayed wood microsites in my study.

In this study, *Cadophora finlandica* was more frequent in decayed wood microsites. *C. finlandica* is an ascomycetous fungal endophyte that forms ectomycorrhizae very similar to those of *Laccaria bicolor* (Peterson et al., 2008). It is one of only three ECM-forming Helotiales which make up the /meliniomyces

lineage, the others being *Meliniomyces bicolor* and *Rhizoscyphus ericae* (Tedersoo et al., 2010a). These three species are very closely related, and can also form ericoid mycorrhizae (Grelet et al., 2010). It is possible that *C. finlandica* was associated with *Rhododendron* spp. and *Vaccinium* spp., which form a large part of the understory at this site (Craig et al., 2006). Related taxa also include endophytes and saprobes (Tedersoo et al., 2010a) that can live on organic debris in the absence of a host plant (Day and Currah, 2011). Genney et al. (2006) found *C. finlandica* to be twice as frequent as hyphae than as root tips, and while the root tips were limited to the organic horizon, the mycelia were found at all depths. My findings of *C. finlandica* in decayed wood are consistent with the success of this species in organic substrates.

Coarse woody debris has been shown to be effective ECM habitat, regardless of decay stage, and to be equally important in young and old forest stands (Elliot et al., 2007). Consequently, in addition to detecting differences among microsites for individual ECM fungal species, I expected to see unique ECM fungal communities inhabiting different forest microsites (Iwanski and Rudawska, 2007; Tedersoo et al., 2003). Tedersoo et al. (2008) and Goodman and Trofymow (1998) documented a clear difference in the frequency and abundance of ECM root tips found in logs versus forest floors. In addition, Tedersoo et al. (2003) were able to determine microsite preferences for some ECM fungal lineages and other fungal orders in a spruce forest: /amphinema-tylospora (Order Atheliales) and /tomentella-thelephora (Order Thelephorales) mycorrhizae were strongly

associated with CWD, while members of the Helotiales and Agaricales were prominent in mineral soils. Tedersoo et al. (2008) noted that ECM fungal species that were dominant in wood were also common in the forest floor. Perhaps my inability to detect profound differences in the ECM fungal community among microsites was related to limiting my analyses to the occurrence, as opposed to abundance, of fungal OTUs, a restriction related to the analysis method (pyrosequencing) I used.

Another major difference in approach between my study and most of those cited above is that I studied the occurrence of ECM hyphae, not ECM roots. The few studies that have compared the occurrence of ectomycorrhizae and the extramatrical hyphae of the same fungal species, found a correlation in distribution for some ECM fungi, but not others (Izzo et al., 2005, 2006; Kjøller, 2006; Genney et al., 2006). Therefore, by detecting less of a difference in hyphae communities among microsites than expected, this study underscores the different perspective offered by studying fungal mycelia.

4.4.2 Ectomycorrhizal fungal communities differed among plots

Plots were different from each other in terms of fungal community composition based on pyrosequencing reads, subsequent OTUs, and once taxa were named to fungal order and ECM lineage combined or by ECM lineage alone. The ECM lineages that differed among plots (e.g. /cenococcum, /hygrophorus, /sebacina,

/wilcoxina) may be structured by spatial organization, niche preferences, and/or host plant relationships.

Investigations of the ECM community structure among plots at the scale measured in this study are uncommon, as most experiments have sought to detect the spatial organization among ECM fungi over centimeters to meters within plots (reviewed by Lilleskov et al., 2004; Genney et al., 2006; Pickles et al., 2010; Tedersoo et al 2006). My plots were approximately 1 km apart; Izzo et al. (2005, 2006) compared plots in a temperate fir forest that were separated by 200 m to over 1.5 km. They found that while some members of the ECM fungal root tip community, including *Cenococcum geophilum* and *Wilcoxina* spp., were widespread and detectable at virtually all plots, most ECM fungal taxa were detectable as root tips in only one plot (Izzo et al., 2005). Interestingly, while it has been documented that the distribution of ECM root tips and their extramatrical hyphae are rarely the same (Genney et al. 2006; Kjøller, 2006), when spores and hyphae were sampled in a subsequent study, *C. geophilum* and *Wilcoxina* spp. remained dominant, but the overall differences among plots were diminished (Izzo et al., 2006). By sampling fungal hyphae in this study, I would expect to have found a uniform distribution of these taxa, even among my distantly-spaced plots.

Cenococcum geophilum and *Wilcoxina* spp., when present, are typically widely distributed with high frequencies among samples (Izzo et al., 2005; Jones et al.,

2010). Therefore the distribution of the /Cenococcum (absent at C), and /wilcoxina lineages (absent at A) in my study is unexpected. The overall detection of *Cenococcum* with pyrosequencing technology is poor (Kauserud et al., 2011), and I have noted that this technique is biased against members of the Pezizomycotina (i.e. *Wilcoxina*; Chapter 2), but this does not explain the absence of these taxa from one plot. ECM fungi exhibit variable patchiness (Lilleskov et al., 2004; Pickles et al., 2010), but I do not believe our findings, at least for /cenococcum to be related to patch size. *C. geophilum* forms small (i.e. less than 300 cm^3) patches (Genney et al., 2006) of low biomass (Lilleskov et al., 2004) evenly dispersed throughout the soil and root systems (Genney et al., 2006; Lilleskov et al., 2004). *Cenococcum geophilum* appears to be well-suited to numerous site conditions (Dickie et al., 2007 and references therein), and its ecology is not related to N-supply (Avis and Charvat, 2005). In contrast, *Wilcoxina* is able to more efficiently mobilize N than is *Cenococcum* (Jones et al. 2009); its absence from a plot with high available ammonium is curious. Interestingly, in pot experiments, colonization by *Wilcoxina mikolae* of its preferred host is interrupted by the presence of ericoid plants (Kohout et al., 2011). While all plots at Sicamous Creek have an ericoid shrub understory, it is possible that there is a unique interaction between these plant taxa and *Wilcoxina* hyphae in plot A. While this final point is highly speculative, I can conclude that the distribution of *Cenococcum* and *Wilcoxina* hyphae at this site does not appear to be explained by spatial organization or niche preference.

There is increasing evidence that host species and the presence of ericaceous plants affect ECM fungal community assemblage (Kohout et al., 2011), and these may have affected the distribution of /hygrophorus, and /sebacina, respectively, at our site. The ECM lineage /hygrophorus was both frequent and abundant at plot A. The growth of *Hygrophorus* spp. mycelium is strongly dependent on undisturbed forest, (Bradbury et al. 1998) and the root tips (Rosling et al., 2003) and DNA (Taylor et al. 2010) of some species are found mostly in organic layers. However, I was not able to relate this to specific nutrient requirements at my site. Most ECM fungi are host-generalists, meaning that they associate with a number of different plant host taxa, however *Hygrophorus olivaceoalbus* is host-specific on spruce (Taylor et al. 2010; Toljander et al., 2006). In addition, ECM fungal communities are strongly structured by host tree diversity (Ishida et al., 2007), especially along natural elevation gradients (Kernaghan and Harper, 2001). I observed a shift in the mixture and density of host trees with a higher fir/spruce ratio, and more gaps, with increased elevation (J. Walker, personal observation). Therefore, it is possible that the dominance of the /hygrophorus lineage at plot A is related to an abundance of spruce hosts as compared to the other plots, especially the highest elevation plot (C). I detected members of the lineage /sebacina at all plots, although they were most frequent at plot B. ECM fungi in the Sebacinaceae (a fungal family that contains members with many trophic habits) are common in open mixed woods, and in forests (Taylor et al. 2010; Tedersoo et al., 2006), and are important associates of ericaceous plants and orchids (Smith and Read, 2008). Both of these plant types occur in forests at

Sicamous Creek (Lloyd and Inselberg, 1998) and it is possible that their unique abundance or distribution within the understory plant community of plot B was overlooked. I conclude that host plant relationships contribute to the structure of /hygrophorus and /sebacina communities detected as hyphae at this site.

The ECM fungal community, as both root tips (Toljander et al., 2006) and hyphae (Nilsson et al., 2005), changes along natural biotic and abiotic gradients in temperate forests. I speculate that the natural elevation gradient, increasing from Plot A to C, and a related shift in the understory plants (Lloyd and Inselberg, 1998) and overstory trees (J. Walker, personal observation), contributed to the structure of the ECM fungal community. I also detected changes among plots in chemical properties such as pH, form of inorganic N and soil organic matter chemistry, which are known to be strong drivers of mycorrhizal community structure in forest soils (Toljander et al. 2006; Parrent et al., 2006; Lilleskov et al., 2002). However, I could not distinguish relationships between changes in the ECM community and changes in soil abiotic properties, nor any shifts related to spatial limitations.

4.4.3 Enzyme activity was strongly influenced by plot properties

The activity of most of the fungal enzymes was structured by plot properties, which included a shift in the ECM fungal community, and a change in soil chemical characteristics. Community-level changes in enzyme profiles are not

always observed with a shift in ECM fungal community composition at this scale (Jones et al., 2010, 2011). Enzyme activities, however, often change with soil properties, especially pH (Sinsabaugh et al., 2008) and substrate availability (Geisseler et al., 2010). In this study, there was a relationship between high enzyme activity and low pH, low nitrate, and high non-polar extractives (fats and waxes). Soil pH may partly explain the increase in chitinase and phosphatase activity, since they are known to vary inversely with pH (Sinsabaugh et al., 2008). However, this does not hold for aminopeptidase activity, which increases with soil pH (Sinsabaugh et al., 2008). It is known that the type and availability of N and C can influence N-related microbial enzymes (Geisseler et al., 2010). Therefore, elevated activity of chitinase and aminopeptidase, while normally attributed to the presence of their substrates, could have been in response to insufficient N-supply, and the nature of the dominant C-source (Geisseler et al., 2010). The high carbon fraction as fats and waxes is not a readily accessible form of C for most fungi (Carlile et al., 2001). Most of the enzymes for which I detected high activity (including glucosidase and cellobiohydrolase) respond positively to increased soil organic matter (Sinsabaugh et al., 2008), however this was not characteristic of plot A, where these activities were highest. My detection of high laccase activity where total C and organic matter were lowest is unexpected, since laccase is involved with the breakdown of persistent forms of C (i.e. lignin). Interestingly, others have found that most of the enzymes we tested are correlated with each other, except for laccase, which demonstrates some independence where C is low (Courty et al., 2010). I conclude that the activity of

fungal enzymes was related to plot-level differences in this study, but it was not clear which of the abiotic properties were the strongest drivers of the changes I detected.

The microsite scale was not characterized by a shift in the ECM fungal community. However, the three types of microsite varied considerably in their organic components. For the most part, these were all highest in decayed wood. Despite this, enzyme activity was not strongly influenced by abiotic factors at the microsite scale. Activity of xylosidase, the only enzyme for which I could detect changes at this scale, was lowest in decayed wood, and the reduced activity of this hemicellulose-degrading enzyme in a microsite with high total C was unexpected. Engelmann spruce wood is over 20 % hemicellulose (and almost 75 % cellulose and lignin) (Kirk and Highley, 1973). Though I did not identify the dominant wood-decay fungi in our microsites, I did target only thoroughly decayed wood (i.e. completely without structure due to the action of lignin-degraders). Some of these 'white-rot' fungi are known to target both hemicellulose and lignin in the earliest stages of decay (Blanchette et al., 1989; Pandey and Pitman, 2003). It is likely that high total C in decayed wood microsites in my study was not due to a high level of structural carbohydrates such as hemicellulose. The high proportion of organic matter and elevated polar extractables (which includes polyphenols) suggest that the decayed wood may have been dominated by recalcitrant humic components known to leach from CWD (Spears and Lajtha, 2005; Zalamea et al., 2007).

Available phosphate was also highest in decayed wood microsites, but was not correlated with high phosphatase activity. While phosphatase is required to release phosphate from organic phosphate compounds (e.g. nucleic acids) (Pritsch and Garbaye, 2011), high levels of inorganic P can suppress phosphatase activity (Olander and Vitousek, 2000; Treseder and Vitousek, 2001). In addition, phosphatase production is often induced when N is readily available, since it becomes a limiting factor (Olander and Vitousek, 2000; Treseder and Vitousek, 2001). Phosphatase activity was not expected to change among microsites because it is appears to be redundant across fungi (Courty et al 2006; Pritsch and Garbaye 2011). Therefore, while my phosphatase results are not surprising, it is possible that I would have detected greater physiological differences in the ECM fungal community among microsites if the substrates had differed from each other in their nitrogen properties, since the capacity for N-acquisition varies among ECM fungi (Jones et al., 2009; Kranabetter et al., 2009; Smith and Read, 2008).

I expected that enzyme activities would change with fungal communities among microsites because both ECM and saprotrophic fungi differ in enzymatic activity patterns at the species level (Buée et al., 2007; Courty et al., 2010; Jones et al., 2010, 2011). Buée et al. (2007) found distinct fungal taxa in the different microsites in a study on substrates similar to mine (organic soil, mineral soil, and dead wood in soil). Specifically, woody debris was inhabited by the mycelia of saprotrophs, and by the root tips of the ECM fungal Genera *Tomentella* and

Lactarius. These ECM fungi demonstrated especially high chitinase activity in enzyme assays. However, while enzyme activity varied among fungal species in the same microsite, and among microsites for the same fungal species, the overall community response did not vary among different microsites types (Buée et al., 2007). Therefore, community enzyme profiles may not differ even with a species shift at the microsite scale. In my study, only the occurrence of individual members of non-ECM groups (i.e. saprotrophs) and a few ECM fungal species differed among microsites. It is not surprising that I did not detect a strong a community-level response in decayed wood microsites.

It is possible that fungal communities colonizing decayed wood in forests at Sicamous Creek are no better adapted to breaking down cellulolignin molecules than the fungi inhabiting other microsites. However, I may have failed to detect changes among microsites due to the timing of sampling. For example, Courty et al. (2006, 2010) and Cullings and Courty (2009) found that as the ECM fungal community changed seasonally in response to host carbon provision (Cullings et al., 2008), so did the activity of some enzymes. The hypothesis provided was that ECM fungal enzyme activities respond more to C-status of the host than they do to C-status of the substrate. This could explain why there was no elevated response by the fungal community colonizing decayed wood in my study to any of the assays related to the breakdown of plant cell walls (e.g. laccase, xylosidase, cellobiohydrolase, glucuronidase, and glucosidase). Alternatively, the fungal taxa most capable of responding to carbon-related enzyme assays may

not have been abundant in the season that we sampled the microsites (late summer/early fall). Others have found that different ECM fungi vary in abundance on root tips, and in metabolic activity across the seasons (Buée et al., 2005; Courty et al., 2006; 2010).

One general problem with comparing my results with those from root tip assays is that my samples, although dominated by the hyphae of ECM fungal taxa, contain a mixture of fungi contributing to the overall assay. To date, research has exclusively documented variation among individual ECM fungal species (Buée et al., 2005, 2007; Courty et al., 2006, 2010). Nevertheless, I expected that the entire assemblage of fungi colonizing distinct microsite types would respond differently when assayed for enzymes that break down a variety of substrates.

4.4.4 A greater number of ECM taxa were identified using pyrosequencing than in my previous studies

The most abundant fungal taxon overall was the ECM lineage /amphinema-tylospora, which was uniformly abundant at all plots. This is consistent with my earlier findings that *Tylospora* spp. had colonized root tips in all forest plots (Chapter 3), and that DNA of *Amphinema* and *Tylospora* spp. together contributed almost 50 % to the overall fungal community in mesh bags from adjacent clearcuts (Chapter 2). Closer inspection of Table C.3 (this Chapter) shows that *Tylospora* spp. contribute much more than *Amphinema* spp. to the abundance of the /amphinema-tylospora lineage detected as hyphae. This

corresponds with the identification of *Tylospora* spp. as an indicator species of the forest community at this site (Chapter 3). Hence, at Sicamous Creek these closely related fungal taxa dominated both the hyphal and root tip communities in the forest. While these taxa are commonly found separately and together in temperate spruce forests as root tips or hyphae (Baier et al., 2006; Landeweert et al., 2003; Rosling et al., 2003), they are rarely among the most abundant taxa (Tedersoo et al., 2008). In addition, identification of both the hyphae and root tip community on a scale such as this study is uncommon.

Although I restricted my analyses to OTUs containing more that 100 pyrosequencing reads, and while only a small percentage of OTUs fell into this category, I was still able to identify the majority of fungal taxa that I detected in my soil samples. However, accumulation curves did not reach an asymptote, demonstrating that many fungal taxa remain undetected despite an enormous sequencing effort. This is common even in other deep sequencing efforts on fungi (Buée et al., 2009; Jumpponen and Jones, 2009; Taylor et al. 2010). The number of reads detected in my study per plot and per 1 g soil or substrate sample (including singletons and doubletons) were similar to other pyrosequencing studies of ECM fungi in forest soils (Buée et al., 2009); however, the number of OTUs, even with a conservative cutoff at 95 % similarity, was high (Buée et al., 2009; Jumpponen and Jones, 2009; Tedersoo et al., 2010). The identity of dominant soil fungi at the higher taxonomic level was also similar to those detected in other studies of temperate forest soils, however the increased

diversity in my study is likely due to our sampling over a much larger area. For example, the studies cited here range from the fungal ecology of single leaves (Jumpponen and Jones, 2009), and the ECM community in 0.1 ha closely-spaced plots (Buée et al., 2009), to one 12 ha forest area (Tedersoo et al., 2010). My 1 ha plots were within 30 ha forest units spaced one km apart; I sampled at both the meter and centimeter scale within these plots. In addition, I encountered a far larger proportion of ECM fungal species among our identified OTUs than were encountered in oak and beech plantations (Buée et al., 2009), but similar to samples from boreal forests subjected to conventional cloning and sequencing (Taylor et al., 2010). The similarity with the boreal forest samples may be because both were from natural stands with similar host species. Most importantly, I identified twice the number of ECM fungal taxa than in my past studies at this site. Finally, this sequencing effort resulted in a broad view of the entire fungal community at Sicamous Creek, including the identity of dominant saprotrophs (e.g. members of the Mortierellales). This study, therefore, presents a comprehensive view of the soil fungal community at many different scales, and one that is unique among these investigations thus far.

5 Conclusion

5.1 Overall analysis of this research and conclusions in light of current research in the field

Current and past research on coarse woody debris (CWD) underscores its importance for many organisms (Arsenault, 2002; Bunnell and Houde, 2010; Craig et al., 2006; Harmon et al., 1986; Jonsson et al., 2005), and confirms that it provides habitat for ectomycorrhizal (ECM) fungi in natural and managed forest stands (Christy et al., 1982; Elliot et al., 2007; Harvey et al., 1979; Olsson et al., 2011; Tedersoo et al., 2003). However, to my knowledge only one published study has specifically investigated the effect of CWD as habitat for ECM fungi in mature forests and regenerating clearcuts (Amaranthus et al. 1994). In addition, no attempts have been made to link the functional contribution of the ECM fungal community in an undisturbed forest to shifts in that community and potential loss of function in disturbed systems, based on the presence or absence of CWD. This was the goal of my thesis.

The moisture-retaining properties of decayed CWD are important for ECM fungi (Harvey et al., 1979). However, few changes have been detected in the chemical and physical characteristics of soil directly below CWD while the wood is still hard (Kayahara et al., 1996; Laiho and Prescott, 1999; Laiho and Prescott, 2004; Spears et al., 2003; Spears and Lajtha, 2004). I was able to establish that the

abiotic properties (e.g. moisture, pH, carbon components, and available mineral nutrients) of decayed wood and of soil beside hard, downed wood differ in distinct ways from nearby mineral soil, especially in clearcuts. The distribution of some ECM fungal species was aligned with these microsite features, providing evidence that both the medium and long-term retention of CWD provides habitat for some ECM taxa in disturbed habitats. These findings help address current gaps in knowledge of specific habitat requirements, as identified by Molina et al. (2010), for the preservation of forest-associated ECM fungi.

Many studies have documented how the ECM fungal community shifts between forests and clearcut sites (Dickie and Reich, 2005; Dickie et al., 2009; Ding et al., 2011; Jones et al., 2003 and references therein; Mah et al., 2001), but very few have demonstrated how ECM physiology is affected by disturbance (Jones et al., 2010). I was able to determine that both ECM community composition and ECM community function shift between forest and clearcut plots. While I could not determine a specific match between an ECM fungal species and an increase or decrease in specific enzyme activity between plots, there was a tendency for the pattern of enzyme activity of the most abundant ECM taxa to shift among plots. For example, the activity profiles of *A. byssoides*, *Wilcoxina* spp., and *Tylospora* spp. in forests contrasted with those of *A. byssoides*, *Wilcoxina* spp., and *T. terrestris* in the clearcuts. From the perspective of regenerating seedlings, the identity and relative activity of these dominant taxa may be important for forest

managers interested in preserving ecosystem function, and/or manipulating the mycorrhizal status of nursery seedlings.

Studies of ECM fungal community composition are increasingly taking into account both ECM root tips and ECM hyphae (Genney et al., 2006; Koide et al., 2005, Kjøller, 2006; Landeweert et al., 2005; Rosling et al., 2004). I combined a thorough investigation of the identity of ECM fungi colonizing spruce root tips, with deep sequencing of the ECM community present as hyphae in a range of substrates in spruce/fir forests and clearcuts. This resulted in a comprehensive view of the of the ECM community that has helped to clarify current questions in the literature about ECM community assemblage, for example those based on inoculum potential and competitive outcomes. I found that the ECM fungal community as living hyphae in clearcuts was more evenly shared among plots than it would have appeared by assessing the ECM root tip community only. While virtually all ECM fungi detected on root tips were detected as hyphae, only a subset of the overall inoculum pool colonized tips at each plot making some ECM taxa appear to exist only in certain plots. This observation is an important reminder that examination of both root tips and hyphae are necessary for a complete view of ECM fungal community structure, and it raises intriguing questions about what shapes the assemblage of mycorrhizae on fine roots in the presence of diverse living inoculum.

Very few studies of ECM fungal communities (Izzo et al., 2005, 2006) have been implemented on multiple temporal and spatial scales. In addition to comparing the ECM fungal community on spruce roots in clearcuts fifteen years post-harvest to that of the community in the first few years after logging, I assessed this community at the microsite and plot scale. My microsite scale samples were approximately one meter from each other, yet the ECM fungal communities were similar in spite of inhabiting different substrates. My plot scale samples were approximately 10 meters from each other, which is a distance beyond which there is unlikely to be autocorrelation in ECM communities (Lilleskov et al., 2004; Pickles et al., 2010). This gave me an ideal view of the plot-level ECM community, and we were able to detect shifts in the ECM fungal community between CWD retention and removal plots, between clearcut and forest plots, and among forest plots separated by one kilometer.

Amaranthus et al. (1994) found that CWD in mature forests provided important habitat for ECM fungi, but that the presence of decayed CWD in regenerating clearcuts did not result in increased frequency or biomass of ECM fruitbodies. In order to achieve the goals set out in my thesis, I first determined that medium-term retention of CWD (hard downed wood) in clearcuts resulted in a shift in the ECM fungal community on root tips, but I was not able to detect a change in the ECM community present as hyphae (Chapter 2). Hard downed wood tended to keep the soil below it cooler than the surrounding clearcut. I also determined that substrate properties were different among microsites that represented the

medium-term (hard downed wood) and long-term (decayed wood) retention of CWD versus the mineral soil, and that a few ECM fungal species were aligned with these different microsites in both forests and clearcuts (Chapters 3 and 4). I established that the ECM fungal community on spruce root tips, and its physiological profile based on depolymerase activity, shifts between forest and clearcut plots (Chapter 3). Finally, I determined that the ECM community present as hyphae in forest soil, and its physiological profile, shifts among forest plots (Chapter 4). I conclude that after fifteen years, the presence of CWD in clearcuts does not result in ECM fungal community structure or function resembling that of the original forest. I could not determine, however, that this resulted in an overall loss of function from the perspective of a regenerating seedling in the clearcut.

5.2 Conclusions based on the hypotheses presented in the Introduction, and the overall contribution of this research

I presented two objectives in the Introduction that were addressed in Chapter 2. The first was to determine if there were differences in ECM fungal community structure between CWD retention and CWD removal plots in clearcuts at Sicamous Creek (Objective 1). The second was to compare ECM fungal communities to those found during initial studies of the ECM fungal community at Sicamous Creek in order to determine whether succession had occurred less than fifteen years after harvest (Objective 2). I predicted that we would not detect a shift in ECM fungal community composition between CWD retention and removal plots due to the scale at which we sampled, and because undecayed

CWD has little influence on the soil conditions below it (Prediction 1). I rejected Hypothesis 1 because we did find a significant difference in the relative abundance of some ECM fungal taxa present on spruce root tips between CWD retention and removal plots. I concluded that while ECM community composition changes as a result of clearcut logging, the outcome can vary with subsequent site manipulation. I speculated that since the logs remained hard and intact, their influence was most likely due to the moderation of soil temperature and moisture, and included these measurements in my subsequent experiments.

To my knowledge, only one group (Avis et al., 2003; Avis and Charvat, 2005) has re-sampled the ECM fungal community more than a decade after treatment or disturbance. Most studies of ECM fungal succession use stands of different ages (i.e., chronosequences) as a proxy for succession (Twieg et al., 2007; Visser et al., 1995). I predicted that we would detect succession in the ECM fungal community on sapling root tips because different fungal species are known to occur soon after disturbance, while others appear later (Twieg et al., 2007) (Prediction 2). I rejected Hypothesis 2 because when I re-sampled the same ECM fungal community 12 years after reforestation, I found that the dominant ECM taxa were still those that were present on nursery seedling roots at outplanting. Other ECM taxa were beginning to colonize the sapling roots, but original forest taxa were still in low abundance on root tips, even though their hyphae were present in the soil. I concluded that it requires more than a decade for the original forest fungi to re-colonize the root systems of high elevation

conifers to any substantial extent after clearcutting. I speculated that if the first species to colonize new fine roots are strong competitors, and well adapted to the new conditions, they may suppress colonization by other native fungi.

The Introduction also contained two objectives that were addressed in Chapter 3. The first was to determine whether the composition and physiological activities of ectomycorrhizae in decayed wood, mineral soil, or adjacent to hard downed wood in clearcuts differed from each other, and if these characteristics were similar to those of ECM fungi in forest microsites (Objective 3). The second objective was to explore the capacity of individual ECM fungal taxa for plasticity among microsites, and to determine if there was evidence of functional complementarity among species that co-occurred in the same microsite (Objective 4). I also measured abiotic properties of the microsite substrates in order to support our speculation in Chapter 2 regarding the reasons for an effect of hard downed wood on the ECM fungal community, in addition to confirming the physical and chemical differences among these substrates.

I predicted that since ECM fungal communities are, at least in part, structured by substrate properties, I would detect a shift in ECM fungal community structure and enzymatic activity among microsites and between plots (Prediction 3). I found evidence that retention of CWD during harvest provided a soil habitat with more forest-like characteristics, and that woody microsites retained or recruited some ECM forest taxa. However, I rejected Hypothesis 3 for microsites because I

detected no overall shifts in the ECM fungal community, nor changes in ECM enzyme profiles, at the microsite scale. My findings at the plot scale supported Hypothesis 3: I detected changes in the ECM fungal community, community enzyme profiles, temperature, pH, Total C, N, and mineral nutrients between forest and clearcut plots. I also predicted that some ECM fungal taxa would exhibit physiological plasticity among microsites, and functional complementarity where they co-occurred. I further predicted that patterns of complementarity among dominant taxa would differ between clearcut and forest plots (Prediction 4). My findings support Hypothesis 4. Among the four dominant ECM fungal taxa that were abundant enough to be tested, I determined that *Tylospora* spp. exhibited phenotypic plasticity among the different microsite types. There was also evidence of functional complementarity among ECM fungal taxa, especially in the forest plots. Interestingly, *Thelephora terrestris* dominated enzyme activity in the clearcuts; its activity was highest for four of eight enzymes. However, while this taxon is among the most abundant ECM fungus in clearcuts at this site, this was not sufficient evidence that ecosystem function was maintained in clearcuts. I concluded that the functional contribution of the ECM fungal community to degradation of soil macromolecules differed among forest and clearcut plots. However, despite the distinct reduction in ECM fungal diversity compared to the adjacent forest, I could not confirm that this resulted in a loss of function in terms of soil organic matter breakdown and acquisition of nutrients for seedlings.

The final objective in the Introduction was addressed in Chapter 4: to determine whether the composition and physiological activities of the fungal community in general, and the ECM community in particular, present as hyphae in the undisturbed forest differed among microsites (Objective 5). I predicted that since fungal communities are structured by substrate properties, the different microsites would be colonized by the hyphae of a different assemblage of fungi, and that I would detect a subsequent shift in fungal enzyme activity among these microsites (Prediction 5). I used advanced pyrosequencing technology to identify members of the fungal community, and found that these substrates were dominated by ECM fungal taxa; however, my findings did not support Hypothesis 5 overall. I was able to show that the entire fungal community, and a few ECM fungal species, differed among microsites, but that fungal enzyme activity varied little at this scale. I determined instead that the fungal community, including the ECM community at the taxonomic levels of lineage and species, varied greatly among the three forest plots, as did the activity of most enzymes. I speculated that the shift in the ECM fungal community among forest plots was due to a number of biotic and abiotic factors related to an elevation gradient among plots. The shift in enzyme activity profiles may have also been driven by the differences in abiotic factors among plots, although I could not determine a clear relationship between them. It is likely that enzyme activity was strongly structured by the clear shift in fungal community composition among plots. I used massively parallel sequencing of fungal DNA to identify fungal taxa present as mycelia in the soil, and was able to taxonomically identify the majority of the pyrosequencing reads,

but I did not exhaustively survey the fungal community at this site. Therefore, I conclude that current technology is not yet able to fully capture the true diversity of the soil fungal community.

5.3 The strengths and limitations of this dissertation

Three important features strengthen this dissertation research. The first strength is that it incorporates multiple temporal and spatial scales. For example, the retention of CWD in clearcuts at the plot scale resulted in an ECM fungal community shift, even over as short a period as 15 years. This means that forest managers could first expect CWD to affect ECM fungal distribution due to this type of site manipulation in a shorter period of time than anticipated. This led to a focus on microsite-scale community shifts in order to determine how changes in the ECM fungal community might progress over a longer period of time. The inclusion of decayed wood microsites as a proxy for long-term CWD retention increased both the temporal and spatial scales at which I could make observations about the response of the ECM fungal community to CWD retention. A second strength of this research lies in the examination of both structure and function of the ECM community for both root tips and hyphae. Prior knowledge of shifts in ECM communities post-harvest led me to question the associated changes in physiological function since this important ecological outcome is not known. I tested this potential outcome by measuring ECM enzyme activity on root tips in clearcut microsites and comparing it to activity in

the undisturbed forest. I also looked for changes in the ECM community as hyphae among these microsites in the forest. To my knowledge, this combination of approaches has never been done. Finally, an enormous strength of this research lies in the use of next generation sequencing technology. With this, I attempted to uncover the immense diversity not detectable with conventional techniques without costly and lengthy cloning and Sanger sequencing or other molecular identification methods (Taylor et al., 2010).

By contrast, the limitations of this research are directly related to the attempt at such a broad scope, and to the use of new technology. For example, the first limitation is due to my efforts to characterize the physiology of as many members of the ECM fungal community as possible on root tips (i.e., mycorrhizae were loaded into microplates so that all rare taxa from each seedling were represented, and the remainder of the wells filled with root tips of the most abundant taxa). I did this so that I could measure the response of the entire community among microsites, but in attempting to do so, I had insufficient replication for thoroughly investigating the response of individual dominant taxa. This limited my ability to test intriguing questions about ECM fungal ecology. Although I was able to detect patterns of functional complementarity among dominant ECM fungal taxa in forest and clearcuts plots, I could not test for complementarity among individual ECM fungal taxa in microsites, nor could I examine plasticity among microsites for more than a few dominant ECM fungal taxa.

A second limitation is a consequence of the technology I used to identify fungal hyphae. Most molecular studies of fungal communities are limited by the quality and quantity of sequence information in public databases; my study was also limited by the short sequence reads generated by the pyrosequencing technology available at the time (100 to 300 bp). Therefore, I used a conservative approach to naming the fungal taxa; in most cases, I felt confident in naming fungi at the level of ECM lineage or fungal order. This did not reduce my ability to detect variation among microsites and plots for the ECM fungal community because I also tested for differences using OTUs, a taxonomic level equivalent to species. It is only the final name attached to each OTU that may be inaccurate.

A final limitation of this research is one that is common to many ecological studies of soil fungi: limited power to detect changes in the community due to low replication and enormous natural variation among samples. I attempted to adjust for this by accepting a statistical p-value of 0.1, and by considering community data as independent variables. This means that I could examine the response of the entire community and the response of individual taxa without adjusting the p-value for multiple variables. Therefore, while I considered non-independent data such as the soil abiotic properties in light of their Bonferroni-corrected p-values, this was not the case for the occurrence or relative abundance of individual ECM taxa. This resulted in my statistically significant acceptance of responses by these taxa among microsites and plots that may otherwise have not been considered valid.

5.4 Future research directions emerging from the work in this dissertation

Two exciting research directions emerge from this dissertation, both associated with current questions in the literature surrounding ECM community ecology. The first relates to theories of community assemblage, and incorporates the idea of priority effects, and competition for space on root tips. The second is centered on ECM community function, and includes the physiological plasticity of individual ECM fungal taxa, and functional complementarity among ECM fungi.

In Chapter 2, I found that the ECM fungal community on spruce saplings still resembled the community present on nursery-grown seedlings at outplanting. I speculated that the ECM fungi on nursery seedlings were excluding other native fungi. I proposed that this was due to a 'priority effect', where the first colonists continue to dominate space on the root tips. I also proposed that the ECM fungi colonizing the nursery seedlings were better adapted to warmer, drier, and nutrient-depleted conditions in the clearcut, and were able to outcompete any residual forest fungi. This hypothesis could be tested explicitly by comparing the ECM fungal community on the operationally planted sapling roots with those colonizing newly planted non-mycorrhizal bioassay seedlings. If priority effects play an important role in this system, one would expect to see different ECM fungi colonizing the non-mycorrhizal seedlings as compared to those resident on the sapling roots. In addition, clearcut and forest fungi known to be present at

Sicamous Creek, and amenable to culturing (e.g. *Thelephora terrestris* and *Laccaria bicolor*), could be used in microcosm experiments to test for priority effects and relative competitive outcomes using the approach of Kennedy and Bruns (2005) and Kennedy et al. (2007).

In Chapter 4, I had only enough replication to test the phenotypic plasticity and functional complementary of a few clearcut and forest ECM fungal taxa. This limitation was not based on having collected too few ECM fungal taxa, only in assaying too few of the most dominant ones. In order to thoroughly test the phenotypic plasticity of individual ECM taxa, and the functional complementarity among dominant ECM fungi, future research must focus on performing enzyme assays on only the dominant ectomycorrhizae in forests and clearcuts. Bioassay seedlings planted in forest and clearcuts are an effective way to sample live ectomycorrhizae, and the experiment can be designed so that the microplate wells are filled with multiple replicates of the same dominant taxa per seedling. I have already identified these taxa in this dissertation. Sufficient replication in enzyme assays will help to determine their functional contribution in the intact forest, and this can be compared with that of ECM fungal taxa in the disturbed system. This information can then be used to assess whether community function is maintained or lost in clearcuts when ECM fungal community diversity is lost.

The outcome of both of these additional experiments, taken together, would be valuable for forest managers, and could reinforce a major conclusion by some researchers (Dickie et al., 2009; Jones et al., 2003; Kranabetter, 2004). These authors contemplate that, while the loss of species in the clearcuts results in a decline in fungal diversity, there may be no reduction in seedling establishment or growth if the remaining ECM fungi species are still able to provide sufficient nutrient resources to the seedling. If the clearcut-dominant fungi continue to outcompete the forest-associated fungi, and yet the patterns and/or level of enzyme activity in the forests and clearcuts remain similar, this hypothesis will gain support.

5.5 Potential management application based on this research

My research shows that hard, downed wood retained on clearcut blocks provides habitat for some ECM fungi, and results in a shift in the ECM fungal community in less than fifteen years. Long-term retention of downed wood, assessed in this thesis by using decayed wood microsites, also provides habitat for certain ECM fungi. However, the retention of downed wood post-harvest does not result in ECM community structure or function resembling that of the original forest in only fifteen years. I conclude, therefore, that retention of CWD on clearcut blocks is valuable in the short and long term, if only to provide diverse habitat for regenerating seedlings and their ECM fungal symbionts.

References

Aerts, R. 2002. The role of various types of mycorrhizal fungi in nutrient cycling and plant competition. In: van der Heijden MGA and IR Sanders (Eds.) Mycorrhizal Ecology, 117-133. Springer-Verlag, Berlin.

Agerer, R. 1987-2002. Colour atlas of ectomycorrhizae. Einhorn-Verlag Eduard Dietenberger, Schwäbisch Gmünd, Germany.

Agerer, R. 2001. Exploration types of ectomycorrhizae: A proposal to classify ectomycorrhizal mycelial systems according to their pattern of differentiation and putative ecological importance. Mycorrhiza 11, 107-114.

Allmér, J., Stenlid, J., Dahlberg, A., 2009. Logging-residue extraction does not reduce the diversity of litter-layer saprotrophic fungi in three Swedish coniferous stands after 25 years. Can. J. For. Res. 39, 1737-1748.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J.H., Zhang, Z., Miller, W., Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST – a new generation of protein database search programs. Nucleic Acids Res. 25, 3389-3402.

Amaranthus, M., Trappe, J.M., Bednar, L., Arthur, D. 1994. Hypogeous fungal production in mature Douglas-fir forest fragments and surrounding plantations and its relation to coarse woody debris and animal mycophagy. Can. J. For. Res. 24, 2157-2165.

Amend, A.S., Seifert, K.A., Bruns, T.D. 2010. Quantifying microbial communities with 454 pyrosequencing: does read abundance count? Mol. Ecol. 19, 5555-5565.

Anderson, M.J. 2005. PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. Department of Statistics, University of Aukland, New Zealand.

Anderson, I.C., Cairney, J.W.G. 2007. Ectomycorrhizal fungi: exploring the mycelial frontier. FEMS Microbiol. Rev. 31, 388-406.

Arocena, J.M., Glowa, K.R., Massicotte, H.B., Lavkulich, L. 1999. Chemical and mineral composition of ectomycorrhizosphere soils of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) in the Ae horizon of a Luvisol. Can. J. Soil Sci. 79, 25-35.

Arocena, J.M., Glowa, K.R., Massicotte, H.B. 2001. Calcium-rich hypha encrustations on *Piloderma*. Mycorrhiza 10, 209-215.

Arsenault, A. 2002. Managing Coarse Woody Debris in British Columbia's Forests: A Cultural Shift for Professional Foresters? In: Laudenslayer Jr., W.F., Shea, P.J., Valentine, B.E., Weatherspoon, C.P., Lisle, T.E. (Eds.), Proceedings of the Symposium on the Ecology and Management of Dead Wood in Western Forests. Gen. Tech. Rep. PSW-GTR-181. pp. 869-878. USDA Forest Service.

Aucina, A., Rudawska, M., Leski, T., Skridaila, A., Riepsas, E., Iwanski, M. 2007. Growth and mycorrhizal community structure of *Pinus sylvestris* seedlings following the addition of forest litter. Appl. Environ. Microbiol. 73, 4867-4873.

Avis, P.G., McLaughlin, D.J., Dentinger, B.C., Reich, P.B. 2003. Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of Russula spp. in a temperate oak savannah. New Phytol. 160, 239-253.

Avis, P.G., Charvat, I. 2005. The response of ectomycorrhizal fungal inoculum to long term increases in nitrogen supply. Mycologia 97: 329-337.

Avis, P.G., Branco, S., Tang, Y., Mueller, G. 2010. Pooled samples bias fungal community descriptions. Mol. Ecol. Res. 10, 135-141.

Baier, R., Ingenhaag, J., Blaschke, H., Göttlein, A., Agerer, R. 2006. Vertical distribution of an ectomycorrhizal community in upper soil horizons of a young Norway spruce (*Picea abies* [L.] Karst.) stand of the Bavarian Limestone Alps. Mycorrhiza 16, 197-206.

Baldrian, P. 2006. Fungal laccases – occurrence and properties. FEMS Microbiol. Rev. 30, 215-242.

Berg, Å, Ehnström, B., Gustafsson, L., Hallingbäck, T., Jonsell, M., Weslien, J. 1994. Threatened Plant, Animal, and Fungus Species in Swedish Forests: Distribution and Habitat Associations. Cons. Biol. 8, 718-731.

Berg, M.P., Ellers, J. 2010. Trait plasticity in species interactions: a driving force of community dynamics. Evol. Ecol. 24, 617-629.

Berthrong, S.T., Jobbágy, E.G., Jackson, R.B. 2011. Global meta-analysis of soil exchangeable cations, pH, carbon, and nitrogen with afforestation. Ecol. Appl. 19, 2228-2241.

Blanchette, R.A., Abad, A.R., Farrell, R.L., Leathers, T.D. 1989. Detection of lignin peroxidase and xylanase by immunocytochemical labeling in wood decayed by basidiomycetes. Appl. Env. Microbiol. 55, 1457-1465.

Botton, S., van Heusden, M., Parsons, J.R., Smidt, H., van Straalen, N. Resilience of microbial systems towards disturbances. Crit. Rev. Microbiol. 32, 101-112.

Buée, M., Vairelles, D., Garbaye, J. 2005. Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (*Fagus sylvatica*) forest subjected to two thinning regimes. Mycorrhiza 15, 235-245.

Buée, M., Courty, P.E., Mignot, D., Garbaye, J. 2007. Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. Soil Biol. Biochem. 39, 1947-1955.

Buée, M., Reich, M., Murat, C., Morin, E., Nilsson, R.H., Uroz, S., Martin, F. 2009. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytol. 184, 449-456.

Bunnell, F., Houde, I. 2010. Down wood and biodiversity – implications to forest practices. Environ. Rev. 18, 397- 421.

Burke, D.J., Weintraub, N., Hewins, C.R., Kalisz, S. 2011. Relationship between soil enzyme activities, nutrient cycling and soil fungal communities in a northern hardwood forest. Soil Biol. And Biochem. 43, 795-803.

Bütler, R. Patty, L., Le Bayon, R-C, Guenta, C., Schlaepfer, R. 2007. Log decay of Picea abies in the Swiss Jura Mountains of central Europe. For. Ecol. Man. 242: 791-799.

Bruns, T.D. 1995. Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. Plant Soil 170, 63-73.

Caldwell, B.A. 2005. Enzyme activities as a component of soil biodiversity: a review. Pedobiologia 49, 637-644.

Carlile, M.J., Watkinson, S.C., Gooday, G.W. 2001. The Fungi 2nd Ed. Academic Press, London.

Chao, A. 2005. Species richness estimation. In: Balakrishnan, N., Vidakovic, B. (Eds.), Encyclopedia of Statistical Sciences. pp. 7909-7916. Wiley, New York.

Christy, E.J., Sollins, P., Trappe, J.M. 1982. First-year survival of Tsuga heterophylla without mycorrhizae and subsequent ectomycorrhizal development on decaying logs and mineral soil. Can. J. Bot. 60, 1601-1605.

Cline, M.L., France, R.C., Reid, C.P.P. 1987. Intraspecific and interspecific growth variation of ectomycorrhizal fungi at different temperatures. Can. J. Bot. 65, 869-875.

Colwell, R.K. 2009 EstimateS: Version 8.2. Statistical estimation of species richness and shared species from samples. http://purl.oclc.org/estimates

Cooke, R.C., Whipps, J.M. 1993. Ecophysiology of Fungi. Blackwell Scientific Publications, Oxford.

Courty, P.-E., Pritsch, K., Schloter, M., Hartmann, A., Garbaye, J. 2005. Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. New Phytol. 167, 309–319.

Courty, P.-E., Pouysegur, R., Buée, M., Garbaye, J. 2006. Laccase and phosphatase activities of the dominant ectomycorrhizal types in a lowland oak forest. Soil Biol. Biochem. 38, 1219-1222.

Courty, P.-E., Bréda, N., Garbaye, J. 2007. Relation between oak tree phenology and the secretion of organic matter degrading enzymes by *Lactarius quietus* ectomycorrhizas before and during bud break. Soi. Bio. Biochem. 39, 1655-1663.

Courty, P.-E., Franc, A., Pierrat, J.-C., Garbaye, J. 2008. Temporal changes in the ectomycorrhizal community in two soil horizons of a temperate oak forest. Appl. Env. Microbiol. 74, 5792-5801.

Courty, P.-E., Franc, A., Garbaye, J. 2010. Temporal and functional pattern of secreted enzyme activities in an ectomycorrhizal community. Soil Biol. and Biochem. 42, 2022-2025.

Craig, V.J., Klenner, W., Feller, M.C., Sullivan, T.P. 2006. Relationships between deer mice and downed wood in managed forests of southern British Columbia. Can. J. For. Res. 36, 2189-2203.

Cullings, K., Ishkhanova, G., Henson, J. 2008. Defoliation effects on enzyme activities of the ectomycorrhizal fungal Suillus granulatus in a Pinus contorta (lodgepole pine) stand in Yellowstone National Park. Oecologia 158, 77-83.

Cullings, K., Courty, P.-E. 2009. Saprotrophic capabilities as functional traits to study functional diversity and resilience of ectomycorrhizal community. Oecologia 161, 661-664.

Day, M.J., Currah, R.S. 2011. Role of selected dark septate endophyte species and other hyphomyces as saprobes on moss gametophytes. Botany 89, 349-359.

Deacon, J. 2006. Fungal Biology 4th Ed. Blackwell Publishing, Maine.

Dentinger, B.T.M., McLaughlin, D.J. 2006. Reconstructing the Clavariaceae using nuclear subunit rDNA sequences and a new genus segregated from *Clavaria*. Mycologia 98**,** 746-762.

Dickie, I. A., Reich, P.B. 2005. Ectomycorrhizal fungal communities at forest edges. J. Ecol. 93, 244–255.

Dickie, I.A. 2007. Host preferences, niches, and fungal diversity. New Phytol. 174, 233-235.

Dickie, I.A., Richardson, S.J., Wiser, S.K. 2009 Ectomycorrhizal fungal communities and soil chemistry in harvested and unharvested temperate *Nothofagus* rainforests. Can. J. For. Res. 39, 1069-1079.

DIckie, I.A. 2010. Insidious effects of sequencing errors on perceived diversity in molecular surveys. New Phytol. 188: 916-918.

Dickie, I.A., Fukami, T., Wilkie, J.P., Allen, R.B., Buchanan, P.K. 2012. Do assembly history effects attenuate from species to ecosystem properties? A field test with wood-inhabiting fungi. Ecol. Letters 15, 133-141.

Ding, Q., Liang, Y., Legendre, P., He, X-h., Pei, K-q, Du, X-j., and Ma, K-p. 2011. Diversity and composition of ectomycorrhizal community on seedling roots: the role of host preference and soil origin. Mycorrhiza 12, 669-680.

Druebert, C., Lang, C., Valtanen, K., Polle, A. 2009. Beech carbon productivity as driver of ectomycorrhizal abundance and diversity. Plant Cell Env. 32, 992-1003.

Dufrêne, M., Legendre, P. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67, 345-366.

Dumbrell, A.J., Nelson, M., Helgason, T., Dytham, C., Fitter, A.H. 2010 Relative roles of niche an neutral processes in structuring a soil microbial community. ISME J. 4, 337-345.

Elliott, J.C., Smith, J.E., Cromack Jr., K., Chen, H., McKay, D. 2007. Chemistry and ectomycorrhizal communities of coarse wood in young and old-growth forests in the Cascade Range of Oregon. Can. J. For. Res. 37, 2041-2051.

Feller, M. 1997. Coarse Woody Debris in Forests: An Overview of the Coarse Woody Debris Study and the Sicamous Creek Study Area. In: Hollstedt, C., Vyse, A. (Eds.), 1997. Sicamous Creek Silvicultural Systems Project: Workshop Proceedings. April 24-25, 1996. Kamloops, British Columbia. Work. Pap. 24/1997. Res. Br., B.C. Min. For., Victoria, B.C., Canada.

Fukami, T., Dickie, I.A., Wilkie, J.P., Paulus, B.C., Park, D., Roberts, A., Buchanan, P.K., Allen, R.B. 2010. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. Ecol. Letters 13, 675-684.

Gagné, A., Jany, J-L., Bousquet, J., Khasa, D.P. 2006. Ectomycorrhizal fungal communities of nursery-inoculated seedlings outplanted on clear-cut sites in northern Alberta. Can. J. For. Res. 26, 1684-1694.

Gardes, M., Bruns, T.D. 1993. ITS primers with enhanced specificity for Basidiomycetes – application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113-118.

Geisseler, D., Horwath, W.R. 2008. Extracellular protease activity in soil in response to different concentrations of nitrogen and carbon. Soil Biol. Biochem. 40, 3040-3048.

Geisseler, D., Horwath, W.R., Joergensen, R.G., Ludwig, B. 2010. Pathways of nitrogen utilization by soil microorganisms – a review. Soil Biol. Biochem. 42, 2058-2067.

Genney, D.R., Anderson, I.C., Alexander, I.J. 2006. Fine-scale distribution of pine ectomycorrhizas and their extrametrical mycelium. New Phytol. 170, 381-390.

Goodman, D.M., Durall, D.M., Trofymow, J.A., Berch, S.M. (Eds.), 1996. A manual of concise descriptions of North American Ectomycorrhizae. Mycologue Publications, 3A1-3A5. Sydney, B.C., Canada.

Goodman, D.M., Trofymow, J.A. 1998. Distribution of ectomycorrhizas in microhabitats in mature and old-growth stands of Douglas-fir on southeastern Vancouver Island. Soil Biol. Biochem. 30, 2127-2138.

Grelet, G.-A., Johnson, D., Vrålstad, T., Alexander, I.J., Anderson, I.C. 2010. New insights into the mycorrhizal *Rhizoscyphus ericae* aggregate: spatial structure and co-colonization of ectomycorrhizal and ericoid roots. New Phytol. 188, 210-222.

Grønflaten, L.K., Steinnes, E., Örlander, G. 2008. Effect of conventional and while-tree clear-cutting on concentrations of some micronutrients in coniferous soil and plants. Forestry Studies (Metsanduslikud Uurimused) 48, 5-16.

Hagerman, S.H., Jones, M.D., Bradfield, G.E., Gillespie, M., Durall, D.M. 1999. Effects of clear-cut logging on the diversity and persistence of ectomycorrhizas at a subalpine forest. Can. J. For. Res. 29, 124-134.

Hagerman, S.H., Durall, D.M. 2004. Ectomycorrhizal colonization of greenhouse-grown Douglas-fir (*Pseudotsuga menziesii*) seedling by inoculum associated with the roots of refuge plants sampled from a Douglas-fir forest in the southern interior of British Columbia. Can. J. Bot. 82, 742-751.

Hannam, K.D., Prescott, C.E. 2003. Soluble organic nitrogen in forests and adjacent clearcuts in British Columbia, Canada. Can. J. For. Res. 33, 1709-1718.

Harmon, M. E., Franklin, J.F., Swanson, F.J., Sollins, P., Gregory, S.V., Lattin, J.D., Anderson, N.H., Cline, S.P., Aumen, N.G., Sedell, J.R., Lienkaemper, G.W., Cromack Jr., K., Cumming, K.W. 1986. Ecology of coarse woody debris in temperate ecosystems. Adv. Ecol. Res. 15, 133-302.

Harvey, A.E., Larsen, M.J., Jurgensen, M.F. 1979. Comparative distribution of ectomycorrhizae in soils of three western Montana forest habitat types. Forest Science 25, 350-358.

Heinonsalo, J., Koskiahde, I., Sen, R. 2007. Scots pine bait seedling performance and root colonizing ectomycorrhizal fungal community dynamics before and during the 4 years after forest clear-cut logging. Can. J. For. Res. 37, 415-429.

Huson, D.H., Auch, A.F., Qi, J., Schuster, S.C. 2007. MEGAN analysis of metagenomic data. Genome Research 17, 377-386. http://ab.inf.uni-teubingen.de/software/megan/

Ishida, T.A., Nara, K., Hogetsu, T. 2007. Host effects on ectomycorrhizal fungal communities: insights from eight host species in mixed conifer-broadleaf forests. New Phytol. 174, 430-440.

Iwánski, M., Rudawska, M. 2007. Ectomycorrhizal colonization of naturally regenerating Pinus sylvestris L. seedlings growing in micro-habitats in boreal forests. Mycorrhiza 17, 461-467.

Izzo A., Agbowo J.A., Bruns, T.D. 2005. Detection of plot-level changes in ectomycorrhizal communities across years in an old-growth mixed-conifer forest. New Phytol. 166, 619-630.

Izzo, A., Nquyen, D.T., Bruns, T.D. 2006. Spatial structure and richness of ectomycorrhizal fungi colonizing bioassay seedlings from resistant propagules in a Sierra Nevada forest: comparisons using two hosts that exhibit different seedling establishment patterns. Mycologia 98, 374-383.

Jones, H.S., Garrett, L.G., Beets, P.N., Kimberly, M.O., Oliver, G.R. 2008. Impacts of harvest residue management on soil carbon stocks in a plantation forest. SSSA J. 6, 1621-1627.

Jones, M.D., Hagerman, S.H., Gillespie, M. 2002. Ectomycorrhizal colonization and richness of previously colonized, containerized *Picea engelmannii* does not vary across clearcuts when planted in mechanically site-prepared mounds. Can. J. For. Res. 32, 1425-1433.

Jones, M.D., Durall, D.M., Cairney, J.G.W. 2003. Ectomycorrhizal fungal communities in young stands regenerating after clearcut logging. New Phytol. 157, 399-422.

Jones, M.D., Grenon, F., Peat, H., Fitzgerald, M., Holt, L., Philip, L.J., Bradley, R. 2009. Differences in 15N uptake among spruce seedlings colonized by three pioneer ectomycorrhizal fungi in the field. Fungal Ecol. 2, 110-120.

Jones, M.D., Twieg, B.D., Ward, V., Barker, J., Durall, D.M., Simard, S.W. 2010. Functional complementarity of Douglas-fir ectomycorrhizas for extracellular enzyme activity after wildfire or clearcut logging. Func. Ecol. 2010, 24, 1139–1151.

Jones, M.D., Phillips, L.A., Treu, R., Ward, V., Berch, S. (In review). Functional responses of ectomycorrhizal fungal communities to long-term fertilization of lodgepole pine (Pinus contorta Dougl. ex Loud. var. latifolia Engelm.) stands in central British Columbia. J. Appl. Soil Ecol.

Jonsson, B.G., Kruys, N., Ranius, T. 2005. Ecology of species living on dead wood – lessons for dead wood management. Silva Fennica 39, 289-309.

Jumpponen, A., Jones, K.L. 2009. Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate Quercus macrocarpa phyllosphere. New Phytol. 184, 438-448.

Katoh, K., Misawa, K., Kuma, K., Miyata, T. 2002. Mafft: A novel method for rapid multiple sequence alignment based on fast fourier transform. Nucl. Acids Res. 30, 3059-3066.

Kauserud, H., Kumar, S., Brysting, A.K., Nordén, J., Carlsen, T. 2011. High consistency between replicate 454 pyrosequencing analyses of ectomycorrhizal plant root samples. Mycorrhiza Published online July 2011 DOI 10.1007/s00572-011-0403-1.

Kayahara, G.J., Klinka, K., Lavkulich, L.M. 1996. Effects of decaying wood on eluviation, podzolization, acidification, and nutrition in soils with different moisture regimes. Environ. Monitor. Assess. 39, 485-492.

Kenkel, N.C. 2006. On selecting an appropriate multivariate analysis. Can. J. plant Sci. 86, 663-676.

Kennedy, P.G., Bruns, T.D. 2005. Priority effects determine the outcome of ectomycorrhizal competition between two *Rhizopogon* species colonizing *Pinus muricata* seedlings. New Phytol. 166, 631-638.

Kennedy, P.G., Hortal, S., Bergemann, S.E., Bruns, T.D. 2007. Competitive interactions among three ectomycorrhizal fungi and their relation to host plant performance. J. Ecol. 95, 1338-1345.

Kennedy, P.G., Peay, K.G., Bruns, T.D. 2009. Root tip competition among ectomycorrhizal fungi: Are priority effects a rule or an exception? Ecology 90, 2098-2107.

Kennedy, P. 2010. Ectomycorrhizal fungi and interspecific competition: species interactions, community structure, coexistence mechanisms, and future research directions. New Phytol. 187, 895-910.

Kennedy, P., Higgins, L.M., Rogers, R.H., Weber, M.G. 2011. Colonization-competition tradeoffs as a mechanism driving successional dynamics in ectomycorrhizal fungal communities. PLoS One 6, 1-10.

Kernaghan, Harper, K.A. 2001. Community structure of ectomycorrhizal fungi across alpine/subalpine ecotone. Ecography 24, 181-188.

Kirk, T.K., Highley, T.L. 1973. Quantitative changes in structural components of conifer woods during decay by white and brown-rot fungi. Phytopathology 63, 1338-1342.

Kipfer, T., Egli, S., Ghazoul, J., Moser, B., Wohlgemuth, T. 2010. Susceptibility of ectomycorrhizal fungi to soil heating. Fungal Biol. 114, 467-472.

Kjøller, R. 2006. Disproportionate abundance between ectomycorrhizal root tips and their associated mycelia. FEMS Microbiol. Ecol. 58, 214-224.

Kohout, P., Sykorová, Z., Bahram, M., Hadincová, V., Albrechtová, J., Tedersoo, L., Vohník, M. 2011. Ericaceous dwarf shrubs affect ectomycorrhizal fungal community of the invasive *Pinus strobus* and native *Pinus sylvestris* in a pot experiment. Mycorrhiza 21, 403-412.

Koide, R.T., Xu, B., Sharda, J. 2005. Contrasting below-ground views of an ectomycorrhizal fungal community. New Phytol. 166, 251-262.

Koide, R.T, Fernandez, C., Petprakob, K. 2011. General principles in the community ecology of ectomycorrhizal fungi. Ann. of For. Sci. 68, 45-55.

Korkama, T., Fritze, H., Pakkanen, A., Pennanen, T. 2007. Interactions between extraradical ectomycorrhizal mycelia, microbes associated with the mycelia and growth rate of Norway spruce (*Picea abies*) clones. New Phytol. 173, 798-807.

Kranabetter, J.M., Friesen, J. 2002. Ectomycorrhizal community structure on western hemlock (*Tsuga heterophylla*) seedlings transplanted from forests into openings. Can. J. Bot. 80, 861-868.

Kranabetter, J.M. 2004. Ectomycorrhizal community effects on hybrid spruce seedling growth and nutrition in clearcuts. Can. J. Bot. 82, 983-991.

Kranabetter, J.M., Durall, D.M., MacKenzie, W.H. 2009. Diversity and species distribution of ectomycorrhizal fungi along productivity gradients of a southern boreal forest. Mycorrhiza 19, 99-111.

Laiho, R., Prescott, C.E. 1999. The contribution of coarse woody debris to carbon, nitrogen, and phosphorus cycles in three Rocky Mountain coniferous forests. Can. J. For Res. 29, 1592-1603.

Laiho, R., Prescott, C.E. 2004. Decay and nutrient dynamics of coarse woody debris in northern coniferous forests: a synthesis. Can. J. For. Res. 34, 763-777.

Landeweert, R., Leeflang, P., Kuyper, T.W., Hoffland, E., Rosling, A., Wernars, K., Smit, E. 2003. Molecular Identification of Ectomycorrhizal Mycelium in Soil Horizons. Appl. Environ. Microbiol. 69, 327-333.

Landeweert, R., Leeflang, P., Smit, E., Kuyper, T. 2005. Diversity of an ectomycorrhizal fungal community studied by a root tips and total soil DNA approach. Mycorrhiza 15, 1-6.

Leake, J.R., Donnelly, D.P., Boddy, L. 2002. Interactions between ecto-mycorrhizal and saprotrophic fungi. In: van der Heijden MGA and IR Sanders (Eds.) Mycorrhizal Ecology, 345-372. Springer-Verlag, Berlin.

Lekberg, Y., Koide, R.T., Rohr, J.R., Aldrich-Wolfe, L. Morton, J.B. 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. Journal of Ecology 95, 95-105.

Lilleskov, E. A., Hobbie, E. A., Fahey, T. J. 2002. Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. New Phytol. 154, 219–231.

Lilleskov, E.A., Bruns, T.D., Horton T.R., Taylor, D.L., Grogan, P. 2004. Detection of forest stand-level spatial structure in ectomycorrhizal fungal communities. FEMS Microbiol. Ecol. 49, 319-332.

Lilleskov, E. A., Hobbie, E. A., Horton T.R. 2011. Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. Fungal Ecol. 4, 174-183.

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, P., Stenlid, J., Finlay, R.D. 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol. 173, 611-620.

Lloyd, D., Angove, K., Hope, G., Thompson, C. 1990. A guide to site identification and interpretation for the Kamloops Forest Region. Land Management Handbook No.23. B.C. Min. For., Victoria, B.C., Canada.

Lloyd, D., Inselberg, A. 1997. Ecosystem mapping for the Sicamous Creek Silvicultural System Research Site. In: Hollstedt, C., Vyse, A. (Eds.), 1997. Sicamous Creek Silvicultural Systems Project: Workshop Proceedings. April 24-25, 1996. Kamloops, British Columbia. Work. Pap. 24/1997. Res. Br., B.C. Min. For., Victoria, B.C., Canada.

Luis, P., Kellner, H., Zimdars, B., Langer, U., Martin, F., Buscot, F. 2005. Patchiness and spatial distribution of laccase genes of ectomycorrhizal, saprotrophic, and unknown basidiomycetes in the upper horizons of a mixed forest cambisol. Microbial Ecol. 50, 570-579.

Mah, K., Tackaberry, L.E., Egger, K.N., Massicotte, H.B. 2001. The impacts of broadcast burning after clearcutting on the diversity of ectomycorrhizal fungi associated with hybrid spruce seedlings in central British Columbia. Can. J. For. Res. 31, 224-235.

Martin, K.J., Rygiewicz, P.T. 2005. Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiol. 5, 28.

Mattson, K.G., Swank, W.T,, Waide, J.B. 1987. Decomposition of woody debris in a regenerating, clear-cut forest in the Southern Appalachians. Can. J. For. Res. 17, 712-721.

Marra, J.L., Edmonds, R.L. 1996. Coarse woody debris and soil respiration in a clearcut on the Olympic Peninsula, Washington, U.S.A. Can. J. For. Res. 26, 1337-1345.

Mayor, J.R., Schuur, E.A.G., Henkel, T.W. 2009. Elucidating the nutritional dynamics of fungi using stable isotopes. Ecol. Letters 12, 171-183.

McCune, B., Grace, J.B. 2002. Analysis of Ecological Communities. MJM Software Design, Gleneden Beach, Oregon.

McCune, B., Mefford, M.J. 2002. PC-Ord. Multivariate Analysis of Ecological Data, Version 5.0. MJM Software Design, Gleneden Beach, Oregon.

McGill, B.J., Enquist, B.J., Weiher, E., Westoby, M. 2006. Rebuilding community ecology from functional traits. Trends Ecol. Evol. 21, 178-185.

Messier, J., McGill, B.J., Lechowcz, M.J. 2010. How do traits vary across ecological scales? A case for trait-based ecology. Ecol. Letters 13, 838-848.

Molina, R., Pilz, D., Smith, J., Dunham, S., Dreisbach, T., O'dell, T., Castellano, M. 2008. Conservation and management of forest fungi in the Pacific Northwestern United States: an integrated ecosystem approach. In: 2001. Fungal Conservation: Issues and Solutions. pp. 19-63. Cambridge University Press, New York.

Molina, R., Horton, T.R., Trappe, J.M., Marcot, B.G. 2010. Addressing uncertainty: How to conserve and manage rare or little-known fungi. Fungal Ecol. 4, 134-146.

Nilsson, L.O., Giesler, R., Bååth, E., Wallander, H. 2005. Growth and biomass of mycorrhizal mycelia in coniferous forests along short natural nutrient gradients. New Phytol 165, 613-622.

Nilsson, R.H., Ryberg, M., Kristiansson, E., Abarenkov, K., Larsson, K.-H., Kõljalg, U. 2006. Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective PLoS One 1, 1-4.

Nilsson, R.H., Bok, G., Ryberg, M., Kristiansson, E., Hallenberg, N., 2009. A software pipeline for processing and identification of fungal ITS sequences. Source Code Biol. Med. 4,1.

Nilsson, R.H. Veldre, V., Hartmann, M., Unterseher, M., Amend, A., Bergsten, J., Kristiansson, E., Ryberg, M., Jumpponen, A., Abarenkov, K. 2010. An open source software package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. Fungal Ecol. 3, 284-287.

Olander, L.P., Vitousek, P.M. 2000. Regulation of soil phosphatase and chitinase activity by regulation of N and P activity. Biogeochem. 49, 175-191.

Olsson, J., Jonsson, B.G., Hjältén, J., Ericson, L. 2011. Addition of coarse woody debris – The early fungal succession on *Picea abies* logs in managed forests and reserves. Biol. Cons. 144, 1100-1110.

Parke, J. L., Linderman, R.G., Trappe, J.M. 1983a. Effect of root zone temperature on ectomycorrhiza and vesicular – arbuscular mycorrhiza formation

in disturbed and undisturbed forest soils of southwest Oregon. Can. J. For. Res. 13, 657-665.

Parke, J. L., Linderman, R.G., Black, C.H. 1983b. The role of ectomycorrhizas in drought tolerance of Douglas-Fir seedlings. New Phytol. 95, 83-95.

Parrent, J.L., Morris, W.F., Vilgalys, R. 2006. CO2 enrichment and nutrient availability alter ectomycorrhizal fungal communities. Ecology 87, 2278-2287.

Parrent, J.L., Peay, K., Arnold, A.E., Comas, L.H., Avis, P., Tuininga, A. 2010. Moving from pattern to process in fungal symbioses: linking functional traits, community ecology and phylogenetics. New Phytol. 185, 882-886.

Peay, K.G., Kennedy, P.G., Bruns, T.D. 2008 Fungal Community Ecology: A hybrid beast with a molecular master. Bioscience 58, 799-810.

Peay, K.G., Garbelotto, M., Bruns, T.D. 2010. Evidence of dispersal limitation in soil microorganisms: Isolation reduces species richness on mycorrhizal tree islands. Ecology 91, 3631-3640.

Peay, K.G., Kennedy, P.G., Bruns, T.D. 2011. Rethinking ectomycorrhizal succession: are root density and hyphal exploration types drivers of spatial and temporal zonation? Fungal Ecol. 4, 233-240.

Pena, R., Offermann, C., Simon, J., Naumann, P.S., Gessler, A. Holst, J., Dannenmann, M., Mayer, H., Kogel-Knaber, I., Rennenberg, H., Polle, A. 2010. Girdling affects ectomycorrhizal fungal (EMF) diversity and reveals functional differences in EMF community composition in a beech forest. Appl. Environ. Microbiol. 76, 1831-41.

Peterson, R.L., Wagg, C., Pautler, M. 2008. Association between microfungal endophytes and roots: do structural features indicate function? Botany 86, 445-456.

Pickles, B.J., Genney, D.R., Potts, J.M., Lennon, J.J., Anderson, I.C., Alexander, I.J. 2010. Spatial and temporal ecology of Scots pine Ectomycorrhizas. New Phytol. 186, 755-768.

Prescott, C.E., Hope, G.D., Blevins, L.L. 2003. Effect of gap size on litter decomposition and soil nitrate concentrations in a high-elevation spruce-fir forest. Can. J. For. Res. 33, 2210-2220.

Pritsch, K., Raidl, S., Marksteiner, E., Agerer, R., Blaschke, H., Schloter, M., Hartmann, A., 2004. A rapid and highly sensitive method for measuring enzyme activities in single mycorrhizal tips using 4-methylumbelliferone-labelled fluorogenic substrates in a microplate system. J. Microbiol. Methods 58, 233-241.

Pritsch, K., Garbaye, J. 2011. Enzyme secretion by ECM fungi and exploitation of mineral nutrients from soil organic matter. Ann. For. Sci. 68, 25-32.

Quinn, G.P., Keough, M.J. 2002. Experimental design and analysis for biologists. Cambridge University Press, Cambridge.

Rajala, T., Peltoniemi, M., Hantula, J., Mäkipää, R., Pennanen, T. 2011. RNA reveals a succession of active fungi during the decay of Norway spruce logs. Fungal Ecol.4, 437-448.

Read, D.J., Perez-Moreno, J. 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance. New Phytol. 157, 475-492.

Read, D.J., Leake, J.R., Perez-Moreno, J. 2004. Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. Can. J. Bot. 82: 1243-1263.

Redding, T.E., Hope, G.D., Fortin, M.-J., Schmidt, M.G., Bailey, W.G. 2003 Spatial patterns of soil temperature and moisture across subalpine forest-clearcut edges in the southern interior of British Columbia. Can. J. Soil Sci. 83, 121-130.

Rineau, F., Courty, P.-E. 2011. Secreted enzymatic activities of ectomycorrhizas as a case study of functional diversity and functional redundancy. Ann. For. Sci. 68, 69-80.

Rosling, A., Landeweert, B.D., Lindahl, B.D., Larsson, K.-H., Kuyper, T.W., Taylor, A.F.S., Finlay, R.D. 2003. Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. New Phytol. 159, 775-783.

Rosling, A., Lindahl, B.D., Finlay, R.D. 2004. Carbon allocation to ectomycorrhizal roots and mycelium colonizing different mineral substrates. New Phytol. 162, 795-802.

Rudawska, M., Leski, T., Trocha, L.K., Gornowicz, R. 2006. Ectomycorrhizal status of Norway spruce seedlings from bare-root forest nurseries. For. Ecol. Man. 236, 375-384.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F. 2009. Introducing MOTHUR: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537-41.

Sinsabaugh, R.L., Saiya-Cork, K., Long, T., Osgood, M.P., Neher, D.A., Zal, D.R., Norby, R.J. 2003. Microbial activity in a *Liquidambar* plantation

unresponsive to CO2-driven increases in primary production. Appl. Soil Ecol. 24, 263-271.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosa, A.R, et al. 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252-1264.

Smith, J.E., Molina, R., Huso, M.M.P., Larsen, M.J. 2000. Occurrence of *Piloderma fallax* in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, U.S.A. Can. J. Bot. 78, 995-1001.

Smith, S.E., Read, D.J. 2008. Mycorrhizal Symbiosis, third ed. Academic Press, London.

Spears, J.D.H., Holub, S.M., Harmon, M.E., Lajtha, K. 2003. The influence of decomposing logs on soil biology and nutrient cycling in an old-growth mixed coniferous forest in Oregon, U.S.A. Can. J. For. Res. 33, 2193-2201.

Spears, J.D.H., Lajtha, K. 2004. The imprint of coarse woody debris on soil chemistry in the western Oregon Cascades. Biogeochem. 71, 163-175.

StatSoft, Inc., 2003. STATISTICA (data analysis software system), version 6.

Stevens, V. 1997. The Ecological Role of Coarse Woody Debris: An Overview of the Ecological Role of CWD in BC Forests. Work. Pap. 30/1997. Res. Br., B.C. Min. For., Victoria, B.C., Canada.

Taylor, D.L., Bruns, T.D. 1999. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Mol. Ecol. 8, 1837-1850.

Taylor, D.L., Herriott, I.C., Stone, K.E., McFarland, J.W., Booth, M.G., Leigh, M.B. 2010. Structure and resilience of fungal communities in Alaskan boreal forest soils. Can J. For. Res. 40, 1288-1301.

Tedersoo, L., Kõljalg, U., Hallenberg, N. Larsson, K-H. 2003. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytol. 159, 153-165.

Tedersoo, L., Suvi, T., Larsson, E., Kõljalg, U. 2006. Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. Mycol. Res. 100, 734-748.

Tedersoo, L., Suvi, T., Jairus, T, Kõljalg, U. 2008. Forest microsite effects on community composition of ectomycorrhizal fungi on seedling of Picea abies and Betula pendula. Environ. Microbiol. 10, 1189-1201.

Tedersoo, L., Gates, G., Dunk, C.W., Lebel, T., May, T.W., Kõljalg, U., Jairus, T. 2009. Establishment of ectomycorrhizal fungal community on isolated Nothofagus cunnighamii seedlings regenerating on dead wood in Australian temperate forests: does fruit-body type matter? Mycorrhiza 19, 403-416.

Tedersoo, L., May, T.W., Smith, M.E. 2010a. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20, 217-263.

Tedersoo, L., Nilsson, R.H., Abarenkov, K., Jairus, T., Sadam, A., Saar, I., Bahram, M., Bechem, E., Chuyong, G., Kõljalg, U. 2010b. 454 Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological bias. New Phytol. 188, 291-301.

Toljander, J.F., Eberhardt, U., Toljander, Y.K., Paul, L.R., Taylor, A.F.S. 2006. Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. New Phytol. 170, 873-884.

Treseder, K.K., Vitousek, P.M. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. Ecology 84, 946-954.

Twieg, B.D., Durall. D.M., Simard, S.W. 2007. Ectomycorrhizal fungal succession in mixed temperate forests. New Phytol. 176, 437-447.

Vellend, M. 2010. Conceptual synthesis in community ecology. Quart. Rev. Biol. 85, 183-206.

Visser, S. 1995. Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytol. 129, 389-401.

Vyse, A. 1997. The Sicamous Creek Silvicultural Systems Project: How the project came to be and what it aims to accomplish. In: Hollstedt, C., Vyse, A. (Eds.), 1997. Sicamous Creek Silvicultural Systems Project: Workshop Proceedings. April 24-25, 1996. Kamloops, British Columbia, Work Pap. 24/1997. Res. Br., B.C. Min. For., Victoria, B.C., Canada.

Walker, J.K.M., Ward, V., Paterson, C. Jones, M.D. 2012. Coarse woody debris retention in subalpine clearcuts affects ectomycorrhizal root tip community structure within fifteen years of harvest. J. Appl. Soil Ecol. In Press.

Wall, A. 2008. Effect of removal of logging residue on nutrient leach and nutrient pools in the soil after clearcutting in a Norway spruce stand. For. Ecol. Man. 3256, 1372-1383.

Wallander, H., Nilsson, L.O., Hagerberg, D., Bååth, E. 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. New Phytol. 151, 753-760.

Wallander, H., Göransson, H., Rosengren, U. 2004. Production, standing biomass, and natural abundance of ^{15}N and ^{13}C in ectomycorrhizal mycelia collected at different soil depths in two forest types. Oecologia 139, 89-97.

Webster, J., Weber, R.W.S. 2007. Introduction to Fungi. Cambridge University Press, New York.

White, T. J., Bruns, T., Lee, S., Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J., White. T. J (Eds.), PCR Protocols: A Guide to Methods and Applications, pp. 315-322. Academic Press, Inc., New York.

Wilson, A.W., Hobbie, E.A., Hibbett, D.S. 2007. The ectomycorrhizal status of *Calostoma cinnabarinum* determined using isotopic, molecular and morphological methods. Can. J. Bot. 85, 385-393.

Yanai, R.D., Currie, W.S., Goodale, C.L. 2003. Soil carbon dynamics after forest harvest: an ecosystem paradigm reconsidered. Ecosystems 6, 197-212.

Zalamea, M., Gonzáles, G., Ping, C.-L., Michaelson, G. 2007. Soil organic matter dynamics under decaying wood in a subtropical wet forest: effect of tree species and decay stage. Plant Soil 289, 173-185.

Appendices

Appendix A Chapter 2 supplemental figures and tables

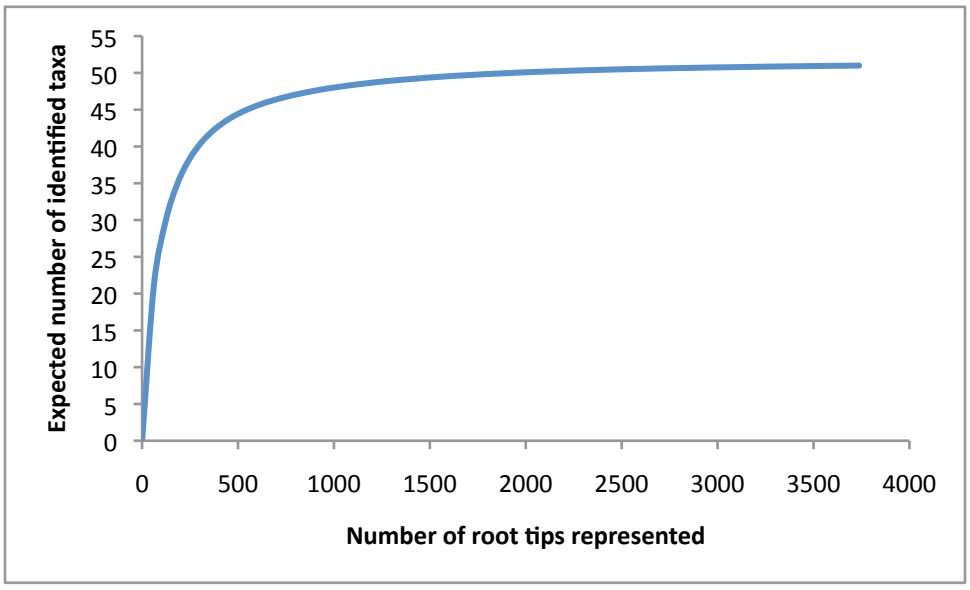


Figure A.1 Number of ECM taxa identified from sapling root tips.

Table A.1 NCBI identities, taxonomic placement, and final names of fungal OTUs identifiable beyond phylum from sapling root tips, clustered at 95% similarity.

Final name of this OTU for analyses[1]	# of root tips represented by this OTU	Best NCBI database accession number and identity[2]	# of bases (%match)[2]	Taxonomic placement by MEGAN
Thelephora terrestris	982	EU427323 Thelephora terrestris	539/539 (100%)	Thelephora terrestris
Alloclavaria purpurea[3]	710	DQ486690 Alloclavaria purpurea	553/559 (99%)	Alloclavaria purpurea
Amphinema byssoides	314	AY219839 Amphinema byssoides	484/486 (99%)	Amphinema byssoides
Tylospora asterophora	206	AF052556 Tylospora asterophora	523/530 (98%)	Tylospora asterophora
Inocybe jacobi	109	AM882710 Inocybe jacobi	501/505 (99%)	Inocybe jacobi
Lactarius sp. 2	100	EF685058 Lactarius deliciosus var	483/615 (78%)	Lactarius
Inocybe sp. 4	86	EF218770 uncultured ECM Inocybe	632/641 (98%)	Inocybe
Nolanea sp.[4]	84	DQ494680 Nolanea strictia	522/554 (94%)	Nolanea cf. verna
Agaricomycetes 1[5]	79	DQ486690 Alloclavaria purpurea	480/529 (90%)	Agaricales
Lactarius sp. 1	75	EF685056 Lactarius deliciosus var	593/651 (91%)	Lactarius
Pezizomycetes[5]	72	EU597015 uncultured ECM Ascomycota	367/384 (95%)	Pezizomycetes
Inocybe sp. 3	53	AY750157 Inocybe lacera	592/595 (99%)	Inocybe
Russula aeruginea	52	AF418612 Russula aeruginea	558/569 (98%)	Russula aeruginea
Cenococcum geophilum[6]	44	AY940649 Cenococcum geophilum	354/354 (100%)	Dothideomyceta
Tylospora sp. 5	42	AF052556 Tylospora asterophora	366/376 (97%)	Tylospora
Helotiaceae 2[5]	42	DQ320128 Cadophora finlandica	377/386 (97%)	Helotiaceae
Hygrophorus sp.	41	DQ490631 Hygrophorus pudorinus	282/308 (91%)	Hygrophorus monticola
Amphinema sp.	41	EF493272 Amphinema byssoides	329/355 (92%)	Amphinema
Ceratobasidiaceae[5]	40	EU218894 Ceratobasidium sp	416/434 (95%)	Fungi
Agaricomycetes 3[5]	36	EU668296 uncultured Sistotrema	148/165 (89%)	Agaricomycetes incertae sedis
Cortinariaceae 2[5]	36	U56043 Dermocybe idahoensis	443/449 (98%)	Cortinariaceae
Pyronemataceae 3[5]	35	DQ069000 Wilcoxina mikolae	319/345 (92%)	Pyronemataceae
Pyronemataceae 2[5]	33	AY880942 Wilcoxina mikolae	372/398 (93%)	Pyronemataceae
Inocybe sp. 1	29	AM882710 Inocybe jacobi	300/339 (88%)	I. jacobi
Pyronemataceae 4[5]	29	EU668262 uncultured Wilcoxina	517/551 (93%)	Pyronemataceae4
Tricholomataceae[5]	28	AY097046 uncultured Tricholoma sp	537/547 (98%)	Tricholomataceae
Pyronemataceae 1[5]	27	DQ069000 Wilcoxina mikolae	555/601 (92%)	Pyronemataceae

Table A.1 cont'd

Final name of this OTU for analyses[1]	# of root tips represented by this OTU	Best NCBI database accession number and identity[2]	# of bases (%match)[2]	Taxonomic placement by MEGAN
Helotiales[5]	26	AY729937 Gyoerffyella rotula	284/342 (83%)	Helotiales
Pyronemataceae 5[5]	25	AY219841 Wilcoxina mikolae	297/311 (95%)	Pyronemataceae
Helotiaceae 1[5]	25	DQ320128 Cadophora finlandica	460/479 (96%)	Helotiaceae
Mycena sp. 1[4]	23	EU669223 Mycena tenax	566/601 (94%)	Mycena.
Mycena sp. 2[4]	21	DQ494677 Mycena plumbea	363/381 (95%)	Mycena leptocephala
Tylospora sp. 3	20	AF052559 Tylospora asterophora	298/301 (99%)	Tylospora
Tylospora sp. 1	19	AF052559 Tylospora asterophora	266/275 (96%)	Tylospora
Tylospora sp. 4	19	AF052554 Tylospora asterophora	346/354 (97%)	T. asterophora
Russula sp.	18	DQ367913 Russula decolorans	700/718 (97%)	Russula
Tylospora fibrillosa	17	AF052562 Tylospora fibrillosa	213/220 (96%)	Tylospora fibrillosa
Atheliaceae 2[5]	15	FJ152541 uncultured ECM Atheliaceae	354/410 (86%	Amphinema diadema
Entoloma sp.	15	AB301602 Entoloma rhodopolium	754/856 (88%)	E. caeuleopolitum
Pyronemataceae 6[5]	11	DQ069000 Wilcoxina mikolae	413/452 (91%)	Pyronemataceae e 6
Sebacina sp.	10	AF202728 Sebacina vermifera	166/171 (97%)	Sebacina vermifera
Cortinariaceae 1[5]	10	AY669585 Cortinarius olivaceofuscus	477/507 (94%)	Cortinariaceae
Inocybe sp. 2	7	AM882751 Astrosporina alpigenes	515/525 (98%)	Inocybe sp. 2
Thelephora sp.	7	DQ822828 Thelephora terrestris	704/737 (95%)	Thelephorales
Galerina sp.[4]	6	AJ585471 Galerina lubrica	460/471 (97%)	Galerina.
Atheliaceae 1[5]	5	DQ469289 Piloderma olivaceum	429/496 (86%)	Atheliaceae
Piloderma sp.	4	AY010281 Piloderma fallax	528/533 (99%)	Piloderma sp.
Hyaloscyphaceae[4]	4	DQ093752 Chalara microchona	464/471 (98%)	Hyaloscyphaceae
Tylospora sp. 2	3	AF052557 Tylospora asterophora	217/229 (94%)	Tylospora
Hydnaceae[5]	2	AY805624 Sistotrema sernanderi	169/170 (99%)	Sistotrema
Agaricomycetes 2[5]	1	FJ152541 uncultured Atheliaceae	186/188 (98%)	Agaricomycetes

[1]Derived by NCBI match, placement by MEGAN, and morphotyping if required.
[2]Matched to NCBI GenBank via the ITS pipeline with and without uncultured fungi.
[3]Match/cluster @95% molecular similarity with *A. purpurea* fruit body.
[4]Non-ECM taxon and therefore not used for further analyses.
[5]Family, order, or class contains ECM taxa and therefore used for further analyses.
[6]Morphotype description used to support identification.

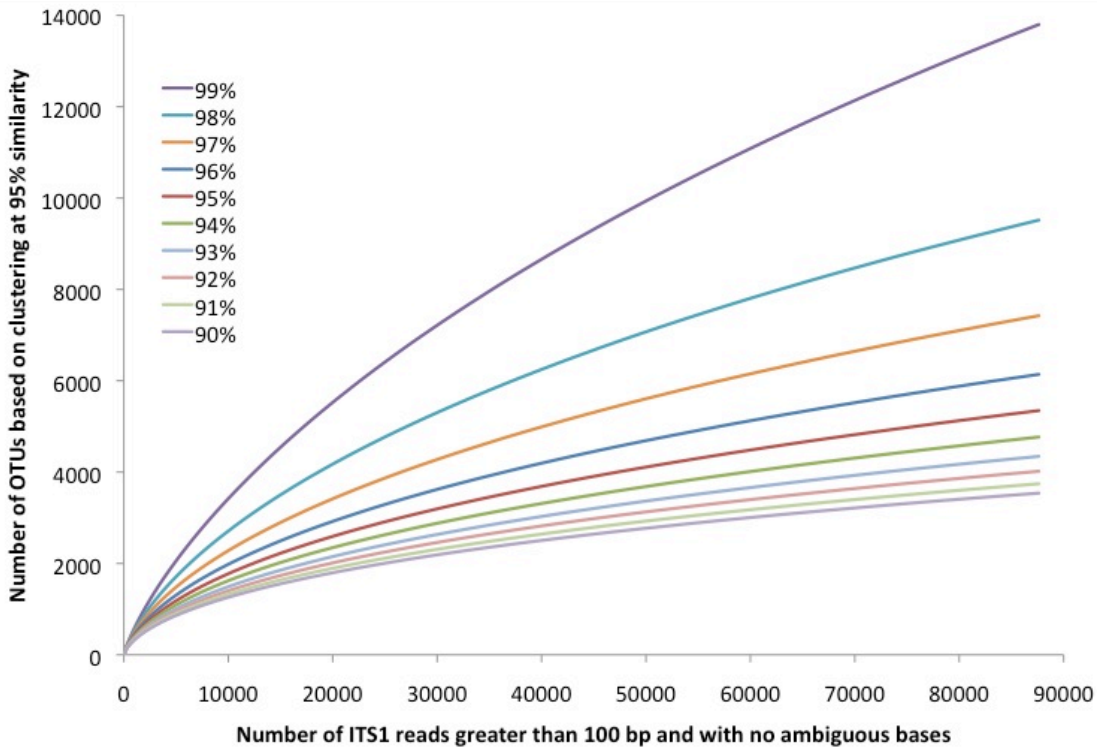


Figure A.2 Number of OTUs identified from mesh bag pyrosequencing ITS1 reads when clustered at molecular sequence similarities ranging from 90 to 99%.

Table A.2 NCBI identities, taxonomic placement, and final names of the most abundant fungal OTUs (i.e. all OTUs with more than 100 reads) from mesh bags when clustered at 95% similarity.

Final name of this OTU for analysis[1,2]	# reads represented by this OTU	Best NCBI database accession number and identity[2]	#of bases matched (%match)[2]	Taxonomic placement by MEGAN
Amphinema byssoides	12712	AY219839 Amphinema byssoides	166/167 (99%)	Amphinema byssoides
Thelephora sp.	2899	EU427330 Thelephora terrestris	196/196 (100%)	Thelephora
Pyronemataceae[4]	2344	DQ069051 Wilcoxina sp	173/174 (99%)	Pyronemataceae
Amphinema sp.	2233	AY838271 Amphinema byssoides	171/176 (97%)	Amphinema
Thelephora sp.	1896	DQ822828 Thelephora terrestris	187/187 (100%)	Thelephora
Thelephora sp.	1796	EU427330 Thelephora terrestris	190/191 (99%)	Thelephora
Thelephora terrestris	1678	EU427323 Thelephora terrestris	205/206 (99%)	Thelephora terrestris
Tylospora sp.	992	AF052556 Tylospora asterophora	172/179 (96%)	Tylospora
Cryptococcus sp.[3]	855	AY040655 Cryptococcus victoriae	130/130 (100%)	Dikarya
Hypholoma sp.[3]	838	AY805610 Hypholoma capnoides	215/215 (100%)	Hypholoma
Cryptococcus sp.[3]	791	AY040655 Cryptococcus victoriae	130/130 (100%)	Dikarya
Pyronemataceae[4]	785	DQ069051 Wilcoxina sp	166/170 (97%)	Pyronemataceae
Amphinema sp.	664	AY838271 Amphinema byssoides	174/176 (98%)	Amphinema
Tylospora sp.	647	AF052556 Tylospora asterophora	171/179 (95%)	Tylospora
Amphinema byssoides	621	AY219839 Amphinema byssoides	161/161 (100%)	Amphinema byssoides
Unknown fungus[3]	583	EF493272 Amphinema byssoides	61/65 (93%)	Unassigned
Amphinema byssoides	566	AY219839 Amphinema byssoides	171/173 (98%)	Amphinema byssoides
Tylospora sp.	562	AF052556 Tylospora asterophora	165/168 (98%)	Tylospora
Unknown fungus[3]	557	EU870071 uncultured Cryptococcus	18/18 (100%)	no hits
Varicosporium elodeae[3]	545	DQ202517 Varicosporium elodeae	173/176 (98%)	Varicosporium elodeae
Amphinema byssoides	522	AY219839 Amphinema byssoides	155/156 (99%)	Amphinema byssoides
Leptodontidium sp.[3]	505	DQ069035 Leptodontidium sp	169/174 (97%)	Fungi
Amphinema sp.	496	AY219839 Amphinema byssoides	184/186 (98%)	Amphinema
Amphinema byssoides	486	AY219839 Amphinema byssoides	170/170 (100%)	Amphinema byssoides
Pholiota sp.[3]	448	AF345654 Pholiota spumosa	240/249 (96%)	Strophariaceae
Unknown fungus[3]	448	AY129287 Pseudeurotium bakeri	90/98 (91%)	no hits
Unknown basidiomycota[3]	464	DQ494702 Xeromphalina campanella	181/221 (81%)	Xeromphalina sp.

Table A.2 cont'd

Final name of this OTU for analysis[1,2]	# reads represented by this OTU	Best NCBI database accession number and identity[2]	#of bases matched (%match)[2]	Taxonomic placement by MEGAN
Amphinema byssoides	408	AY219839 Amphinema byssoides	166/167 (99%)	Amphinema byssoides
Unknown basidiomycota[3]	398	DQ494702 Xeromphalina campanella	106/127 (83%)	Xeromphalina sp.
Thelephora sp.	362	DQ822828 Thelephora terrestris	214/217 (98%)	Thelephora
Mortierellales[3]	353	DQ093723 Mortierella gamsii	143/154 (92%)	Mortierella
Amphinema byssoides	329	AY219839 Amphinema byssoides	174/175 (99%)	Amphinema byssoides
Cryptococcus sp. [3]	324	EU252550 Cryptococcus terricola	170/170 (100%)	Dikarya
Calyptrozyma arxii[3]	319	AJ133432 Calyptrozyma arxii	169/171 (98%)	Calyptrozyma arxii
Laccaria laccata	319	EU819477 Laccaria laccata	254/255 (99%)	Laccaria laccata
Amphinema sp.	308	AY219839 Amphinema byssoides	168/185 (90%)	Amphinema
Amphinema byssoides	303	AY219839 Amphinema byssoides	174/176 (98%)	Amphinema byssoides
Unknown basidiomycota[3]	303	FJ152541 uncultured ECM Atheliaceae	156/157 (99%)	Unassigned
Amphinema sp.	300	AY219839 Amphinema byssoides	111/113 (98%)	Amphinema
Leptodontidium sp. [3]	285	AM262433 Leptodontidium orchidicola	131/135 (97%)	Ascomycota
Psilocybe montana[3]	282	AY129352 Psilocybe montana	196/196 (100%)	Psilocybe montana
Amphinema byssoides	276	AY219839 Amphinema byssoides	173/174 (99%)	Amphinema byssoides
Amphinema byssoides	272	AY219839 Amphinema byssoides	166/168 (98%)	Amphinema byssoides
Amphinema byssoides	272	AY219839 Amphinema byssoides	141/142 (99%)	Amphinema byssoides
Unknown fungus[3]	265	DQ485666 Kappamyces laurelensis	15/15 (100%)	no hits
Amphinema byssoides	263	AY219839 Amphinema byssoides	176/179 (98%)	Amphinema byssoides
Psilocybe montana[3]	262	AY129352 Psilocybe montana	203/204 (99%)	Psilocybe montana
Thelephora terrestris	261	EU427323 Thelephora terrestris	199/200 (99%)	Thelephora terrestris
Unknown basidiomycota[3]	260	FJ152541 uncultured ECM Atheliaceae	166/169 (98%)	Unassigned
Botrytis sp. [3]	257	EU519207 Botrytis elliptica	150/150 (100%)	Sclerotiniaceae
Botrytis sp. [3]	256	EF589862 Botrytis sp	150/150 (100%)	Sclerotiniaceae
Thelephora sp.	247	EU427323 Thelephora terrestris	132/132 (100%)	Thelephora
Helotiales1[4]	246	AY348594 Calycina herbarum	166/167 (99%)	Helotiales
Unknown basidiomycota[3]	245	AF052556 Tylospora asterophora	188/200 (94%)	Root
Mortierella sp. [3]	240	AJ878778 Mortierella humilis	204/208 (98%)	Mortierellales
Helotiales1[4]	239	AM262399 Calycina herbarum	159/161 (98%)	Helotiales

Table A.2 cont'd

Final name of this OTU for analysis[1,2]	# reads represented by this OTU	Best NCBI database accession number and identity[2]	#of bases matched (%match)[2]	Taxonomic placement by MEGAN
Unknown fungus[3]	232	EF493272 Amphinema byssoides	48/50 (96%)	Unassigned
Unknown ascomycota[3]	224	AF481372 ECM root tip 180 Ny2C295	156/158 (98%)	Ascomycota
Unknown fungus[3]	218	AM260905 uncultured fungus	81/84 (96%)	no hits
Physalospora scirpi[3]	216	AB220255 Physalospora scirpi	162/167 (97%)	Physalospora scirpi
Unknown fungus[3]	215	EU680488 uncultured sordariomycete	49/51 (96%)	no hits
Unknown ascomycota[3]	214	AB041243 Allantophomopsis lycopodina	148/162 (91%)	Leotiomycetes
Unknown basidiomycota[3]	211	AF444489 Rhodosporidium toruloides	137/149 (91%)	no hits
Cudonia sp.[3]	206	AF433151 Cudonia lutea	136/140 (97%)	Cudonia
Pyronemataceae[4]	205	DQ069000 Wilcoxina mikolae	172/190 (90%)	Pyronemataceae
Cryptococcus sp.[3]	197	AJ581047 Cryptococcus victoriae	125/126 (99%)	Dikarya
Cryptococcus sp.[3]	191	AJ581047 Cryptococcus victoriae	129/129 (100%)	Cryptococcus
Cladophialophora sp.[3]	190	EF016377 Cladophialophora minutissima	156/156 (100%)	Cladophialophora
Mortierella sp.[3]	190	AJ878778 Mortierella humilis	149/150 (99%)	Mortierellales
Pseudotomentella tristis	185	AJ889968 Pseudotomentella tristis	217/220 (98%)	Pseudotomentella tristis
Allantophomopsis sp.[3]	182	AB041243 Allantophomopsis lycopodina	160/164 (97%)	Ascomycota
Unknown fungus[3]	181	EU529971 uncultured ECM fungus	109/109 (100%)	no hits
Unknown fungus[3]	181	DQ661898 uncultured fungus	194/196 (98%)	no hits
Unknown fungus[3]	179	AF145324 Cryptococcus aerius	18/18 (100%)	no hits
Mortierella sp.[3]	174	AJ541799 Mortierella sp	168/169 (99%)	Mortierella sp.
Mrakia sp.[3]	169	AJ866977 Mrakia frigida	163/165 (98%)	Mrakia
Unknown ascomycota[3]	160	AY969405 uncultured ascomycete	155/159 (97%)	Unassigned
Unknown fungus[3]	158	AY204589 Alatospora acuminata	119/135 (88%)	no hits
Thelephora sp.	158	EU427323 Thelephora terrestris	133/137 (97%)	Thelephora
Amphinema sp.	156	AY838271 Amphinema byssoides	153/158 (96%)	Amphinema
Amphinema sp.	152	AY219839 Amphinema byssoides	102/105 (97%)	Amphinema
Amphinema sp.	151	AY838271 Amphinema byssoides	168/171 (98%)	Amphinema
Pyronemataceae	151	AY880942 Wilcoxina mikolae	152/170 (89%)	Pyronemataceae
Unknown ascomycota[3]	147	AM999726 uncultured fungus	140/141 (99%)	Ascomycota
Amphinema byssoides	143	AY219839 Amphinema byssoides	174/176 (98%)	Amphinema byssoides

Table A.2 cont'd

Final name of this OTU for analysis[1,2]	# reads represented by this OTU	Best NCBI database accession number and identity[2]	#of bases matched (%match)[2]	Taxonomic placement by MEGAN
Unknown basidiomycota[3]	143	FJ152541 uncultured ECM Atheliaceae	159/164 (96%)	Unassigned
Inocybe jacobi	141	AM882710 Inocybe jacobi	162/162 (100%)	Inocybe jacobi
Unknown ascomycota[3]	134	DQ912692 Phoma herbarum	116/116 (100%)	no hits
Mortierella sp.[3]	134	EU240133 Mortierella sp	144/145 (99%)	Mortierella
Rhizoctonia sp.[3]	133	DQ093652 Rhizoctonia sp	164/168 (97%)	Fungi
Tylospora sp.	133	AF052556 Tylospora asterophora	219/233 (93%)	Tylospora
Mortierellales[3]	131	AJ878780 Mortierella hyalina	141/153 (92%)	Mortierella
Unknown fungus[3]	127	AM494587 uncultured Glomus	59/65 (90%)	no hits
Penicillium sp.[3]	127	AF033489 Penicillium kojigenum	150/150 (100%)	Fungi
Thelephora sp.	124	EU427323 Thelephora terrestris	185/186 (99%)	Thelephora
Pyronemataceae[4]	124	DQ069000 Wilcoxina mikolae	169/188 (89%)	Pyronemataceae
Unknown ascomycota[3]	123	AF011327 Cadophora finlandica	124/129 (96%)	Unclassified fungi
Mortierella sp.[3]	122	EF519900 Mortierella alpina	176/176 (100%)	Mortierella
Davidiella sp.[3]	121	EU622923 Davidiella tassiana	165/165 (100%)	Davidiellaceae
Amphinema byssoides	120	AY219839 Amphinema byssoides	174/177 (98%)	Amphinema byssoides
Tylospora sp.	119	AF052556 Tylospora asterophora	169/177 (95%)	Tylospora
Tylospora sp.	118	AF052556 Tylospora asterophora	217/229 (94%)	Tylospora
Unknown fungus[3]	117	AM999599 uncultured fungus	105/107 (98%)	no hits
Xeromphalina sp.[3]	117	GQ890701 Xerophalina sp. PA-2010a	212/224 (95%)	Xeromphalina sp.
Cryptococcus sp.[3]	115	AB032670 Cryptococcus antarcticus	163/163 (100%)	Cryptococcus
Atheliaceae[4]	114	U85794 Athelia epiphylla	163/169 (96%)	Atheliaceae
Thelephora sp.	113	EU427323 Thelephora terrestris	187/196 (95%)	Thelephora
Entoloma sp.	112	EF421108 Entoloma sericeonitidum	208/215 (96%)	Entoloma
Pyronemataceae[4]	111	AY880942 Wilcoxina mikolae	158/175 (90%)	Pyronemataceae
Rhodotorula sp.[3]	111	AB038088 Rhodotorula fujisanensis	131/135 (97%)	Rhodotorula
Sebacina vermifera	109	AM181396 uncultured Sebacinales	175/183 (95%)	Sebacina vermifera
Wilcoxina mikolae	105	AY880942 Wilcoxina mikolae	201/203 (99%)	Wilcoxina mikolae
Hypocrea sp.[3]	105	AM498498 Hypocrea pachybasioides	202/202 (100%)	Fungi-Metazoa
Cladophialophora sp.[3]	104	EF016381 Cladophialophora minutissima	135/136 (99%)	Cladophialophora

Table A.2 cont'd

Final name of this OTU for analysis[1,2]	# reads represented by this OTU	Best NCBI database accession number and identity[2]	#of bases matched (%match)[2]	Taxonomic placement by MEGAN
Amphinema sp.	104	AY838271 Amphinema byssoides	152/155 (98%)	Amphinema
Thelephora sp.	103	EU427323 Thelephora terrestris	161/168 (95%)	Thelephora
Agaricomycetes1[4]	102	DQ309195 uncultured fungus	207/214 (96%)	Agaricomycetes
Unknown ascomycota[3]	102	EF093150 Helotiales sp	147/149 (98%)	Fungi
Unknown ascomycota[3]	101	EF619867 uncultured ascomycete	188/200 (94%)	Unassigned
Unknown fungus[3]	100	AM999727 uncultured fungus	171/171 (100%)	Fungi

[1]NCBI match for information only; placement by MEGAN was used for final name.
[2]Matched to NCBI GenBank via the ITS pipeline with and without uncultured fungi.
[3]Non-ECM taxon or not identified beyond phylum and therefore not used for analyses.
[4]Family, order, or class contains ECM taxa and therefore used for further analyses.

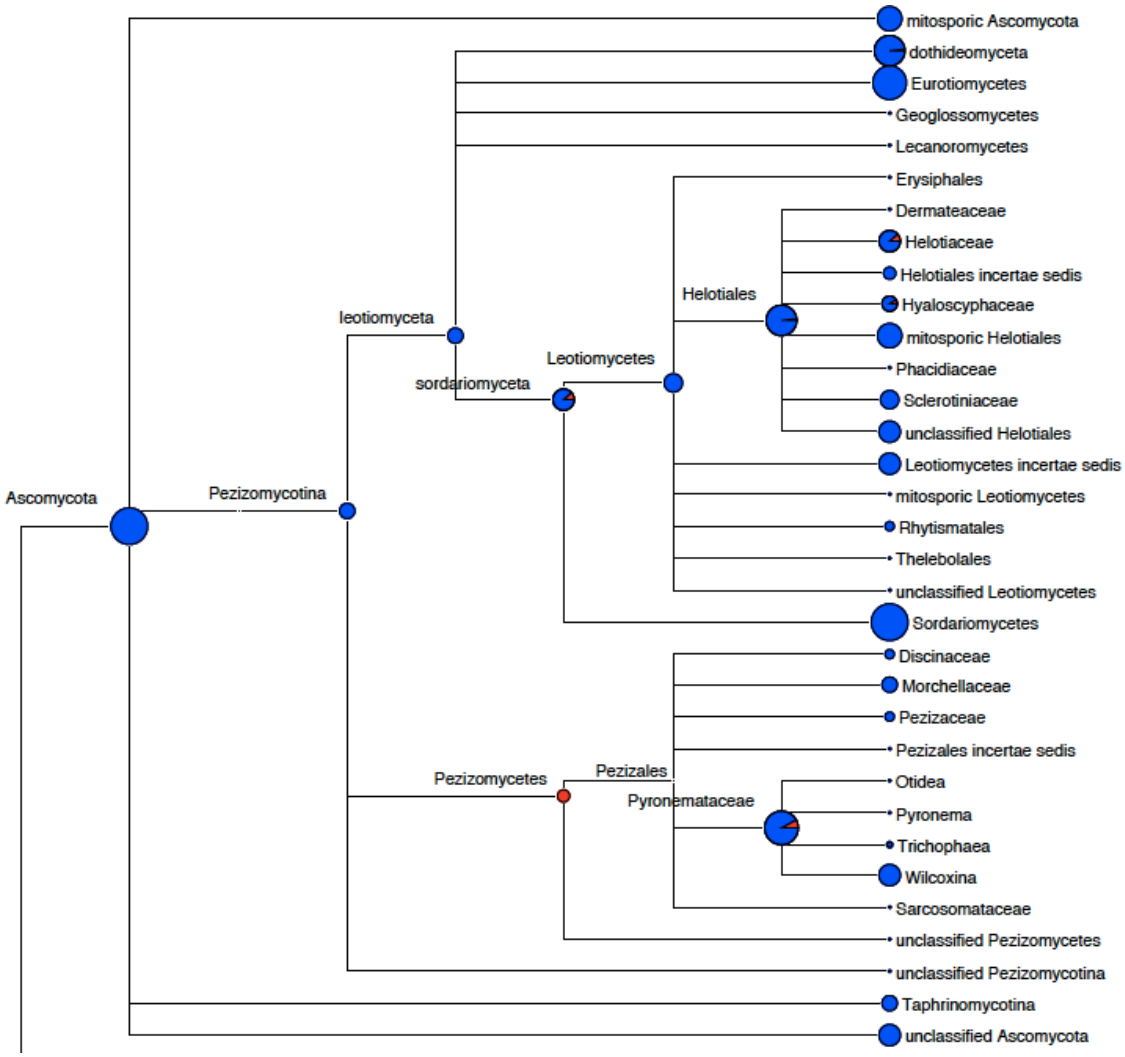


Figure A.3a Comparison of Ascomycota detected on ECM root tips (red) and in mesh bag hyphae (blue).
Size of circle and proportion of pie chart (for taxa represented by both) reflect the absolute number of sequences (root tips) or OTUs (mesh bags) identified. This tree represents the 51 OTUs from 197 root tip sequences, and 2377 of 5347 mesh bag OTUs named to at least the level of Phylum.

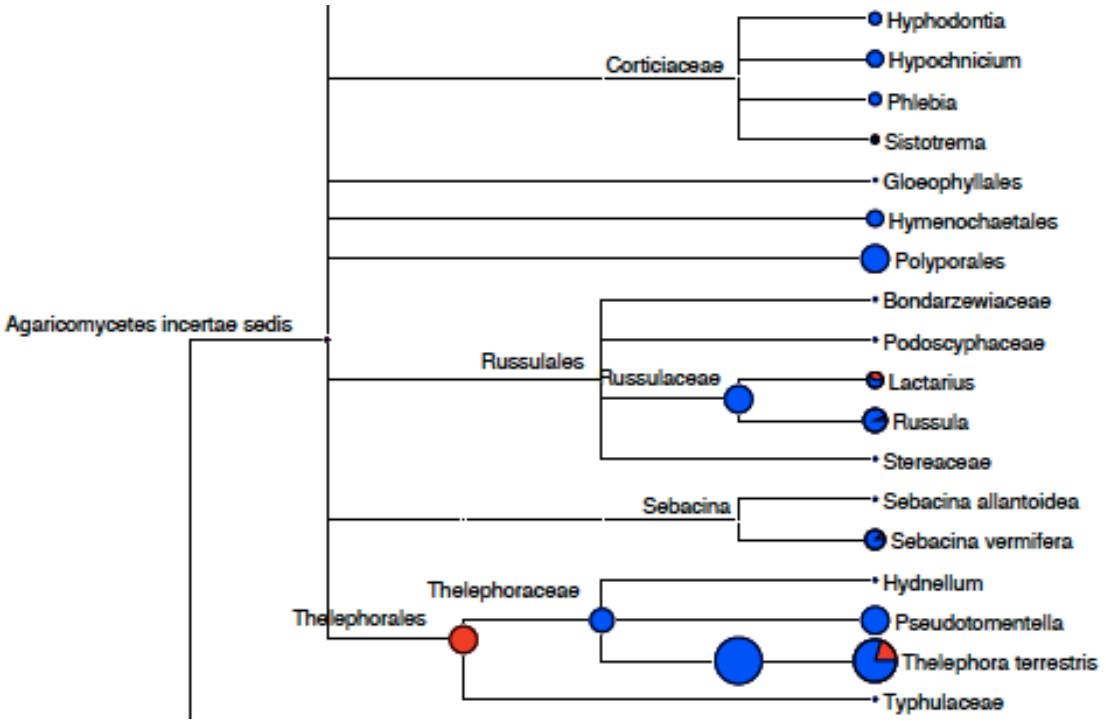


Figure A.3b Comparison of Basidiomycota (Agaricomycetes incertae sedis) detected on ECM root tips (red) and in mesh bag hyphae (blue).
Size of circle and proportion of pie chart (for taxa represented by both) reflect the absolute number of sequences (root tips) or OTUs (mesh bags) identified. This tree represents the 51 OTUs from 197 root tip sequences, and 2377 of 5347 mesh bag OTUs named to at least the level of Phylum.

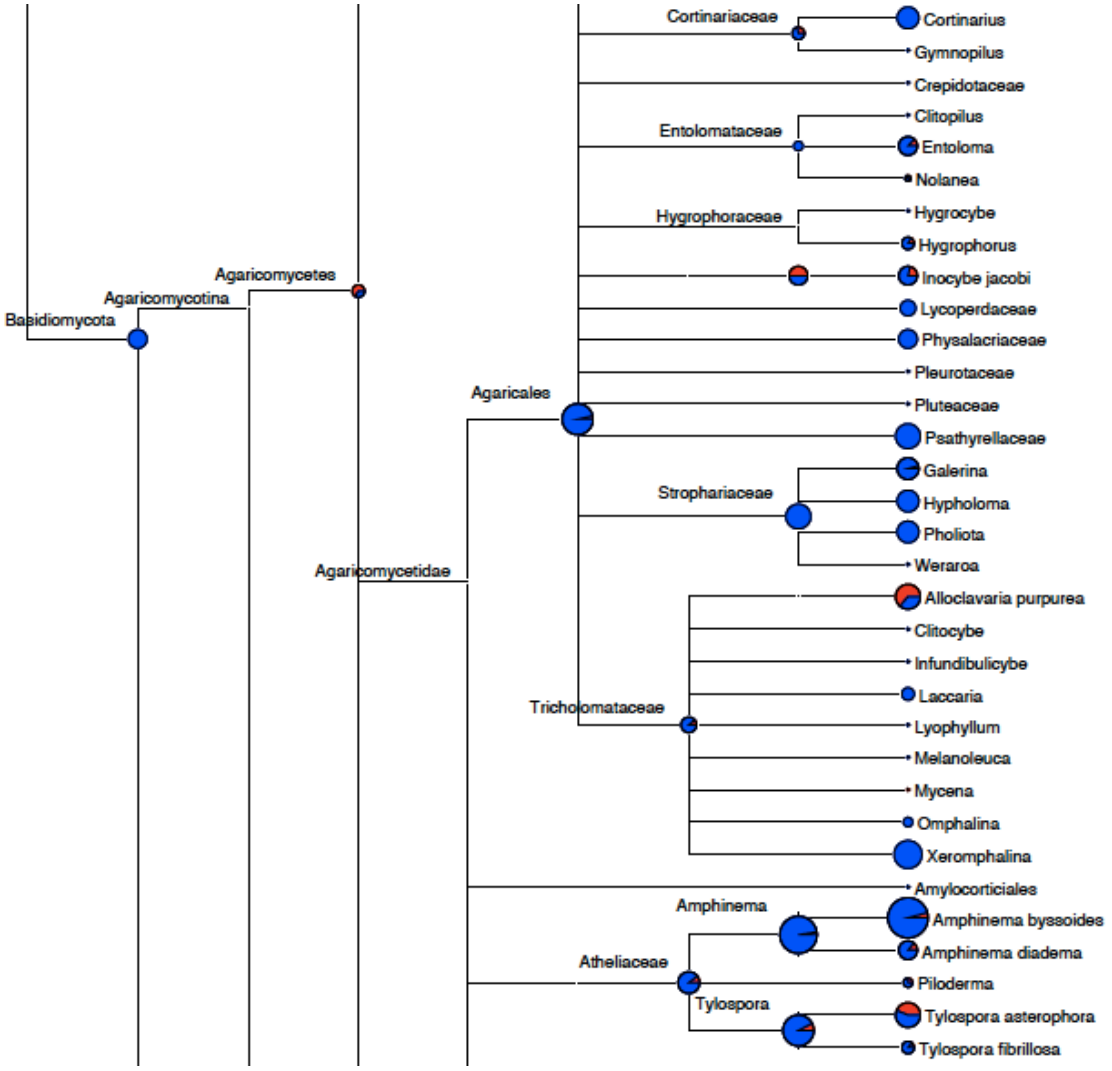


Figure A.3c Comparison of Basidiomycota (Agaricomycetidae) detected on ECM root tips (red) and in mesh bag hyphae (blue).
Size of circle and proportion of pie chart (for taxa represented by both) reflect the absolute number of sequences (root tips) or OTUs (mesh bags) identified. This tree represents the 51 OTUs from 197 root tip sequences, and 2377 of 5347 mesh bag OTUs named to at least the level of Phylum.

Appendix B Chapter 3 supplemental tables

Table B.1 a) Hierarchical univariate ANOVA for Total C per seedling soil sample per microsite, and b) Post-hoc Bonferroni test of Total C at the microsite-level. Bold type is for emphasis only. MANOVA p <0.0001 for plot and microsite.

a)

	Effect	SS	df	MS	F	p
Intercept	Fixed	32.280	1	32.280	85.819	0.011
Block	Random	0.752	2	0.376	1.157	0.318
Plot treatment	Fixed	4.690	2	2.345	7.213	**0.001**
Microsite(Plot)	Fixed	5.517	6	0.919	2.828	**0.013**
Error		40.314	124	0.325		

b)

	Plot	Microsite	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}
1	Forest	Decay		**1.00**	**1.00**	1.00	0.03	1.00	0.26	0.04	1.00
2	Forest	Control	**1.00**		**1.00**	1.00	1.00	1.00	1.00	1.00	1.00
3	Forest	Downed	**1.00**	**1.00**		1.00	0.01	1.00	0.09	0.01	1.00
4	Removal	Decay	1.00	1.00	1.00		**0.13**	**1.00**	0.99	0.18	1.00
5	Removal	Control	0.03	1.00	0.01	**0.13**		**0.50**	1.00	1.00	1.00
6	Removal	Downed	1.00	1.00	1.00	**1.00**	**0.50**		1.00	0.70	1.00
7	Retention	Decay	0.26	1.00	0.09	0.99	1.00	1.00		**1.00**	**1.00**
8	Retention	Control	0.04	1.00	0.01	0.18	1.00	0.70	**1.00**		**1.00**
9	Retention	Downed	1.00	1.00	1.00	1.00	1.00	1.00	**1.00**	**1.00**	

Table B.2 a) Univariate hierarchical ANOVA of per seedling per microsite laccase activity, and b) Post-hoc Bonferroni test of laccase enzyme activity means among microsites. Bold type is for emphasis only.

a)

	Effect	**SS**	**df**	**MS**	**F**	**p**
Intercept	Fixed	3055.561	1	3055.561	2022.534	0.000
Block	Random	3.022	2	1.511	0.861	0.425
Plot treatment	**Fixed**	**142.781**	**2**	**71.391**	**40.701**	**0.000**
Microsite(Plot)	**Fixed**	**25.336**	**6**	**4.223**	**2.407**	**0.031**
Error		217.502	124	1.754		

b)

Plot		Removal			Retention			Forest		
	Microsite	Decay	Control	Downed	Decay	Control	Downed	Decay	Control	Downed
Removal	Decay		**0.021**	**0.957**	1.000	1.000	0.685	0.000	0.000	0.000
	Control	**0.021**		**1.000**	1.000	1.000	1.000	0.447	0.197	0.019
	Downed	**0.957**	**1.000**		1.000	1.000	1.000	0.008	0.003	0.000
Retention	Decay	1.000	1.000	1.000		**1.000**	**1.000**	0.006	0.002	0.000
	Control	1.000	1.000	1.000	**1.000**		**1.000**	0.001	0.000	0.000
	Downed	0.685	1.000	1.000	**1.000**	**1.000**		0.012	0.004	0.000
Forest	Decay	0.000	0.447	0.008	0.006	0.001	0.012		**1.000**	**1.000**
	Control	0.000	0.197	0.003	0.002	0.000	0.004	**1.000**		**1.000**
	Downed	0.000	0.019	0.000	0.000	0.000	0.000	**1.000**	**1.000**	

Table B.3 a) Univariate hierarchical ANOVA of per seedling per microsite observed taxon richness and b) Univariate ANOVA of per plot per block observed taxon richness.

a)

	Effect	SS	df	MS	F	p
Intercept	Fixed	569.482	1	569.482	50.085	0.019
Block	Random	22.741	2	11.370	4.205	**0.034**
Plot treatment	Fixed	116.519	2	58.259	21.548	**0.000**
Microsite(Plot)	Fixed	8.000	6	1.333	0.493	0.804
Error		43.259	16	2.704		

b)

	Effect	SS	df	MS	F	p
Intercept	Fixed	1002.778	1	1002.778	184.184	0.005
Block	Random	10.889	2	5.444	0.405	0.692
Plot treatment	Fixed	169.556	2	84.778	6.306	0.058
Error		53.778	4	13.444		

Table B.4 a) Univariate hierarchical ANOVA of *T. terrestris* relative abundance and b) Post-hoc Bonferroni tests of *T. terrestris* means among plots.

a)

	Effect	SS	df	MS	F	p
Intercept	Fixed	5.40911	1	5.409	23.918	0.038
Block	Random	0.45814	2	0.229	2.139	0.123
Plot treatment	**Fixed**	**2.940**	**2**	**1.470**	**13.725**	**0.000**
Microsite(Plot)	Fixed	0.858	6	0.143	1.336	0.249
Error		10.281	96	0.107		

b)

Plot treatment	Forest	Removal	Retention
Forest		**0.001**	**0.000**
Removal	**0.001**		0.422
Retention	**0.000**	0.422	

Table B.5 a) Multivariate one-way ANOVA for enzyme activity among taxa in forest plots, b) Univariate one-way ANOVA for aminopeptidase activity among taxa in forest plots, and c) Post-hoc Bonferroni test of aminopeptidase activity means among forest taxa.

a).

	Test	Value	F	Effect	Error	p
Intercept	Wilks	0.000	1306.600	6	1	0.021
Taxon	**Wilks**	**0.000**	**37.233**	**12**	**2**	**0.026**

b)

	Effect	SS	df	MS	F	p
Intercept	Fixed	1.525003	**1**	1.525003	32.33501	0.001
Taxon	**Random**	**3.741**	**2**	**1.870453**	**39.65968**	**0.0003**
Error		0.282976	6	0.047163		

c)

Taxon	Abyssoides	Wilcoxina	Tylospora
Abyssoides		1.000	**0.001**
Wilcoxina	1.000		**0.001**
Tylospora	**0.001**	**0.001**	

Appendix C Chapter 4 supplemental tables

Table C.1 Univariate hierarchical ANOVA of a) available P (N=5), and b) polar extractables (N=3) among control soil, downed, and decayed wood microsites, and among plots.

a)

	SS	df	MS	F	p
Intercept	283.8	1	283.8	744.0	0.000
Plot	0.910	2	0.455	1.193	0.315
Microsite(Plot)	5.867	6	0.978	2.563	**0.036**
Error	13.35	35	0.381		

b)

	SS	df	MS	F	p
Intercept	355.4	1	355.4	1987.7	0.000
Plot	1.166	2	0.583	3.261	**0.061**
Microsite(Plot)	6.440	6	1.073	6.002	**0.001**
Error	3.219	18	0.178		

Table C.2 Univariate hierarchical ANOVA of a) minimum daily moisture, and b) maximum daily temperature among control soil, downed, and decayed wood microsites, and among plots in August 2007, 2008, and 2009. N=3.

a)

	SS	df	MS	F	p
Intercept	0.491	1	0.491	417.8	0.000
Year	0.006	2	0.003	2.761	0.103
Plot	0.053	2	0.026	22.63	**0.0001**
Microsite(Plot)	0.144	6	0.024	20.50	**0.00001**
Error	0.014	18	0.001		

b)

	SS	df	MS	F	p
Intercept	2026.1	1	2026.1	14073.2	0.000
Year	7.311	2	3.656	25.3	0.00005
Plot	0.150	2	0.075	0.52	0.606
Microsite(Plot)	16.17	6	3.236	22.4	**0.00001**
Error	1.728	18	0.144		

Table C.3 a-c. Identity of fungal OTUs with at least 100 pyrosequencing reads at forest plot a) A, b) B, and c) C.

a)

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
/amphinema-tylospora1	3842	AF052556 Tylospora asterophora 262 8e-70 185/196 (94%)	UDB002469 Tylospora asterophora 298 3e-80 94.00
/amphinema-tylospora2	2287	AF052562 Tylospora fibrillosa 293 2e-79 164/168 (97%)	DQ068974 Tylospora fibrillosa 278 3e-74 96.47
/amphinema-tylospora3	2553	AY010283 Tylospora fibrillosa 270 3e-72 145/147 (98%)	FJ152490 uncultured Tylospora 287 5e-77 99.37
/amphinema-tylospora4	880	AF052562 Tylospora fibrillosa 256 3e-68 135/137 (98%)	FJ152491 uncultured Tylospora 237 4e-62 97.81
/amphinema-tylospora5	1620	AF052562 Tylospora fibrillosa 281 7e-76 168/174 (96%)	UDB002468 Tylospora fibrillosa 283 7e-76 96.02
/amphinema-tylospora6	985	AY010283 Tylospora fibrillosa 268 1e-71 143/146 (97%)	FJ152490 uncultured Tylospora 281 2e-75 98.74
/amphinema-tylospora7	645	AY219839 Amphinema byssoides 341 9e-94 175/176 (99%)	FJ554364 uncultured Amphinema 326 1e-88 100.00
/amphinema-tylospora8	286	AF052556 Tylospora asterophora 155 7e-38 98/102 (96%)	EU597067 uncultured Tylospora 195 2e-49 100.00
/amphinema-tylospora9	169	AF052564 Tylospora fibrillosa 258 1e-68 148/153 (96%)	EU597068 uncultured Tylospora 300 7e-81 96.65
/amphinema-tylospora10	135	AY010283 Tylospora fibrillosa 208 6e-54 105/105 (100%)	FJ152490 uncultured Tylospora 217 5e-56 100.00
/cenococcum1	112	EU427331 Cenococcum geophilum 274 2e-73 145/146 (99%)	HQ406817 Cenococcum geophilum 263 8e-70 99.32
/cortinarius1	2232	EU821656 Cortinarius traganus 170 1e-42 93/94 (98%)	UDB002406 Cortinarius raphanoides 178 2e-44 95.58
/cortinarius2	1039	DQ367911 Cortinarius caperatus 513 e-145 259/259 (100%)	UDB001079 Rozites caperatus 475 2e-133 99.24
/cortinarius3	179	DQ367911 Cortinarius caperatus 347 2e-95 178/179 (99%)	UDB001079 Rozites caperatus 320 6e-87 98.88

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
/cortinarius4	106	AY040712 Cortinarius betuletorum 224 2e-58 150/160 (93%)	UDB002406 Cortinarius raphanoides 252 2e-66 94.05
/hygrophorus1	19754	EU597040 uncultured ectomycorrhiza Hygrophorus 266 6e-71 148/150 (98%)	UDB000561 Hygrophorus camarophyllus 239 1e-62 97.86
/hygrophorus2	809	AY242852 Hygrophorus cossus 86 9e-17 82/91 (90%)	DQ517418 Hygrophorus olivaceoalbus 95.3 3e-19 86.02
/hygrophorus3	404	EU597040 uncultured ectomycorrhiza Hygrophorus 228 1e-59 122/123 (99%)	UDB000561 Hygrophorus camarophyllus 204 4e-52 98.29
/hygrophorus6	108	EU597040 uncultured ectomycorrhiza Hygrophorus 180 3e-45 137/154 (88%)	UDB000561 Hygrophorus camarophyllus 137 5e-32 96.43
/inocybe1	521	AM882787 Inocybe leptophylla 359 5e-99 207/213 (97%)	FJ553409 uncultured Agaricomycetes 381 3e-105 99.06
/inocybe2	345	DQ367905 Inocybe lanuginosa var 153 2e-37 96/101 (95%)	HQ604315 Inocybe cf jacobi UBC F19047 163 5e-40 96.04
/laccaria1	178	DQ149854 Laccaria nobilis 268 9e-72 138/139 (99%)	FJ845417 Laccaria bicolor 257 3e-68 100.00
/meliniomyces1	138	EF517302 Meliniomyces bicolor 218 8e-57 126/130 (96%)	HQ125186 uncultured fungus 291 4e-78 99.38
/piloderma1	1793	EF493276 Piloderma fallax 319 3e-87 161/161 (100%)	UDB001614 Piloderma fallax 298 2e-80 100.00
/piloderma2	1612	DQ365679 Piloderma fallax 240 2e-63 128/129 (99%)	UDB001739 Piloderma byssinum 217 5e-56 97.64
/piloderma3	3703	DQ365679 Piloderma fallax 355 6e-98 179/179 (100%)	UDB001739 Piloderma byssinum 305 2e-82 98.29
/piloderma4	1569	EF619738 uncultured Piloderma 157 4e-38 114/123 (92%)	DQ377394 uncultured Atheliaceae 187 4e-47 91.37
/piloderma5	249	DQ469288 Piloderma lanatum 305 6e-83 177/182 (97%)	UDB001733 Piloderma 344 3e-94 100.00
/piloderma6	181	AY010280 Piloderma fallax 155 1e-37 108/118 (91%)	HM488561 uncultured Piloderma 289 1e-77 100.00

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
/pseudotomentella 1	975	AF274768 Pseudotomentella mucidula 200 2e-51 104/105 (99%)	UDB001617 Pseudotomentella griseoper.. 228 3e-59 95.74
/pseudotomentella 2	223	AF274768 Pseudotomentella mucidula 168 6e-42 97/101 (96%)	UDB001617 Pseudotomentella griseoper.. 217 6e-56 94.96
/russula-lactarius1	1355	AF418619 Russula emetica 204 2e-52 158/171 (92%)	UDB000914 Russula nana 254 6e-67 91.53
/russula-lactarius2	406	AY336950 Lactarius mairei 291 9e-79 188/199 (94%)	UDB002460 Lactarius musteus 333 8e-91 96.14
/russula-lactarius3	166	AY061733 Russula nauseosa 331 1e-90 184/187 (98%)	UDB001631 Russula odorata 287 6e-77 94.24
/russula-lactarius4	135	AF418621 Russula raoultii 244 2e-64 184/201 (91%)	UDB000914 Russula nana 340 5e-93 97.51
/sebacina1	156	DQ661898 uncultured fungus 287 2e-77 148/149 (99%)	HQ211919 uncultured Sebacina 270 5e-72 99.33
/tomentella-thelephora1	102	EU427323 Thelephora terrestris 392 e-109 198/198 (100%)	HQ406822 Thelephora terrestris 366 8e-101 100.00
Cantharellales1	165	DQ267124 Botryobasidium botryosum 232 5e-61 130/133 (97%)	FJ820672 uncultured fungus 233 5e-61 98.50
Chaetothryiales1	133	EF016377 Cladophialophora minutissima 198 6e-51 121/128 (94%)	GU174353 uncultured fungus 226 9e-59 97.04
Helotiales1	447	AF486132 Phialocephala virens 115 7e-26 106/121 (87%)	HQ021923 uncultured Helotiales 246 7e-65 100.00
Helotiales2	458	DQ914672 Cystodendron sp 285 7e-77 157/160 (98%)	HQ212246 uncultured Helotiales 289 1e-77 99.38
Helotiales3	317	AY781244 ascomycete sp 224 2e-58 144/153 (94%)	HQ845751 Helotiales sp PIMO 265 250 7e-66 94.44
Helotiales4	298	DQ497967 uncultured ectomycorrhizal fungus 381 e-105 192/192 (100%)	HQ157913 Helotiaceae sp VI GK 2010 355 2e-97 100.00
Helotiales5	519	AB211249 uncultured ectomycorrhizal fungus 315 9e-86 180/187 (96%)	HQ157878 Helotiaceae sp V GK 2010 346 1e-94 100.00

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
Helotiales6	172	AY371513 Cadophora sp 224 2e-58 146/157 (92%)	HQ211494 uncultured Leotiomycetes 294 3e-79 99.38
Helotiales7	134	EF434076 uncultured fungus 359 7e-99 184/185 (99%)	HQ157918 Helotiaceae sp III GK 2010 342 1e-93 100.00
Helotiales8	117	EF093148 Helotiales sp 307 2e-83 158/159 (99%)	AY465455 Phialophora sp GS6N4b 289 1e-77 99.37
Leotiomycetes1	222	AY781230 Leptodontidium elatius 210 2e-54 142/153 (92%)	HQ211516 uncultured Leotiomycetes 292 1e-78 98.21
Leotiomycetes2	127	DQ273331 uncultured Pezizomycotina 367 e-101 185/185 (100%)	FJ152527 uncultured Leotiomycetes 342 1e-93 100.00
Mortierellales1	1121	AY969835 uncultured fungus 248 1e-65 125/125 (100%)	HQ022258 uncultured Mortierella 231 2e-60 100.00
Mortierellales2	799	DQ093723 Mortierella gamsii 157 2e-38 134/147 (91%)	DQ309131 uncultured fungus 255 1e-67 98.62
Mortierellales3	354	AJ878780 Mortierella hyalina 137 2e-32 114/127 (89%)	EU806603 uncultured soil fungus 204 4e-52 97.50
Mortierellales4	172	EU806719 uncultured soil fungus 293 3e-79 148/148 (100%)	HQ212330 uncultured Mortierella 209 1e-53 92.72
Mortierellales5	118	AJ878782 Mortierella macrocystis 260 2e-69 149/155 (96%)	HQ212347 uncultured Mortierella 281 2e-75 99.36
Onygenales1	221	AF062787 Oidiodendron pilicola 341 9e-94 175/176 (99%)	HM069414 uncultured fungus 326 1e-88 100.00
Onygenales2	151	AY354254 Oidiodendron scytaloides 244 2e-64 154/163 (94%)	FJ553111 uncultured Ascomycota 298 2e-80 98.81
Pleosporales1	172	AY251083 Venturia hystrioides 260 2e-69 154/159 (96%)	FJ553146 uncultured Venturia 281 2e-75 98.73
Tremellales1	174	AF444350 Cryptococcus terricola 289 3e-78 146/146 (100%)	HQ212245 uncultured Cryptococcus 270 4e-72 100.00
unknown1	2194	AY702742 uncultured fungus from ecm root 111 1e-24 81/88 (92%)	HM069490 uncultured fungus 183 4e-46 99.03

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
unknown2	1476	DQ309201 uncultured fungus 210 3e-54 121/126 (96%)	AY394903 uncultured mycorrhizal fungus 206 1e-52 96.03
unknown3	1165	AF461627 uncultured fungus 56 8e-08 31/32 (96%)	NO HITS FOUND.
unknown4	504	DQ309123 uncultured fungus 34 0.27 26/29 (89%)	GU174299 uncultured fungus 165 1e-40 94.44
unknown5	383	EU622343 Hydnellum ferrugineum 30 4.8 15/15 (100%)	NO HITS FOUND.
unknown6	255	EU292602 uncultured fungus 319 6e-87 179/184 (97%)	FJ660482 uncultured ectomycorrhizal .. 324 4e-88 99.44
unknown7	249	DQ661899 uncultured fungus 107 4e-23 139/164 (84%)	HQ446028 uncultured fungus 268 2e-71 96.91
unknown8	244	DQ233843 uncultured ectomycorrhizal fungus 196 4e-50 119/123 (96%)	HM164554 uncultured fungus 204 4e-52 96.75
unknown9	163	EF521261 uncultured fungus 121 2e-27 90/97 (92%)	HQ126589 uncultured fungus 195 2e-49 96.64
unknown10	146	FJ152543 uncultured ectomycorrhiza Atheliaceae 210 2e-54 115/118 (97%)	FJ152543 uncultured Pezizomycotina 202 1e-51 97.46
unknown11	138	EF434137 uncultured fungus 196 4e-50 136/147 (92%)	HQ126585 uncultured fungus 222 1e-57 93.96
unknown12	124	DQ340311 Hyphodontia hastata 74 4e-13 74/83 (89%)	NO HITS FOUND.
unknown13	218	EU806755 uncultured soil fungus 121 3e-27 73/77 (94%)	FR727722 uncultured fungus 178 4e-44 85.33
unknown14	111	EU554708 uncultured fungus 107 2e-23 102/114 (89%)	GQ223472 uncultured fungus 159 7e-39 92.17
unknown Ascomycota1	307	EF016385 Cladophialophora minutissima 100 3e-21 53/54 (98%)	FN565212 uncultured Ascomycota 202 1e-51 100.00
unknown Ascomycota2	123	AY970069 uncultured ascomycete 182 6e-46 116/124 (93%)	GU366688 uncultured fungus 191 3e-48 94.35

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
unknown Ascomycota3	121	DQ911451 Candida paludigena 216 3e-56 126/129 (97%)	FJ553080 uncultured Saccharomycetes 222 1e-57 97.01
unknown Basidiomycota1	352	DQ481983 uncultured ectomycorrhiza Atheliaceae 212 6e-55 119/123 (96%)	GQ160051 uncultured fungus 211 2e-54 97.56
unknown Basidiomycota2	210	EF434153 uncultured fungus 119 1e-26 173/201 (86%)	FM997946 uncultured Basidiomycota 339 2e-92 97.97
unknown Basidiomycota3	132	DQ200924 Botryobasidium subcoronatum 70 6e-12 63/71 (88%)	FJ820672 uncultured fungus 122 2e-27 80.33

b)

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
/albatrellus1	139	AY558791 Mycolevis siccigleba 200 3e-51 185/209 (88%)	AY558782 Leucogaster sp TK1618 237 7e-62 88.04
/amanita1	121	AY436470 Amanita pseudovaginata 180 2e-45 134/145 (92%)	UDB002321 Amanita friabilis 261 3e-69 100.00
/amphinema-tylospora1	5149	AF052556 Tylospora asterophora 238 1e-62 170/180 (94%)	UDB002469 Tylospora asterophora 274 5e-73 94.02
/amphinema-tylospora2	686	AF052562 Tylospora fibrillosa 291 7e-79 159/163 (97%)	UDB002468 Tylospora fibrillosa 276 1e-73 96.97
/amphinema-tylospora3	3078	AY010283 Tylospora fibrillosa 287 1e-77 150/152 (98%)	AY010283 Tylospora fibrillosa 272 1e-72 98.68
/amphinema-tylospora4	420	AF052562 Tylospora fibrillosa 289 3e-78 159/162 (98%)	AF052562 Tylospora fibrillosa 281 2e-75 98.15
/amphinema-tylospora5	6273	AF052562 Tylospora fibrillosa 287 1e-77 164/169 (97%)	AF052562 Tylospora fibrillosa 283 7e-76 97.04
/amphinema-tylospora6	940	AY010283 Tylospora fibrillosa 270 3e-72 145/147 (98%)	AY010283 Tylospora fibrillosa 261 3e-69 98.64
/amphinema-tylospora7	790	AY219839 Amphinema byssoides 329 3e-90 169/170 (99%)	AY219839 Amphinema byssoides 309 1e-83 99.41

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
/amphinema-tylospora11	2968	AF052556 Tylospora asterophora 327 1e-89 182/185 (98%)	GU550116 Tylospora asterophora 324 4e-88 98.38
/amphinema-tylospora12	4754	AF052556 Tylospora asterophora 293 2e-79 177/184 (96%)	GU550116 Tylospora asterophora 300 8e-81 95.74
/amphinema-tylospora13	875	EF493272 Amphinema byssoides 94 3e-19 61/65 (93%)	FM992887 uncultured Amphinema 268 2e-71 100.00
/amphinema-tylospora14	2120	EF493272 Amphinema byssoides 94 3e-19 61/65 (93%)	FM992887 uncultured Amphinema 285 2e-76 99.37
/amphinema-tylospora15	469	AF052562 Tylospora fibrillosa 260 2e-69 150/155 (96%)	AF052562 Tylospora fibrillosa 257 4e-68 96.77
/amphinema-tylospora16	722	EF493272 Amphinema byssoides 98 2e-20 61/64 (95%)	FM992887 uncultured Amphinema 226 9e-59 97.73
/amphinema-tylospora17	113	AY838271 Amphinema byssoides 299 3e-81 164/167 (98%)	UDB001722 Amphinema byssoides 291 4e-78 98.20
/cenococcum1	545	EU427331 Cenococcum geophilum 234 1e-61 121/122 (99%)	HQ406817 Cenococcum geophilum 220 4e-57 99.18
/cenococcum2	108	EU427331 Cenococcum geophilum 281 6e-76 142/142 (100%)	HQ406817 Cenococcum geophilum 265 2e-70 99.32
/cortinarius5	506	EU525946 Dermocybe sanguinea 200 1e-51 101/101 (100%)	U56054 Dermocybe phoenicea 187 3e-47 100.00
/cortinarius6	252	EU313201 Cortinarius caninus 188 4e-48 98/99 (98%)	UDB002218 Cortinarius anomalus 158 2e-38 94.95
/cortinarius7	184	AY669673 Cortinarius rubricosus 250 3e-66 159/168 (94%)	UDB000169 Cortinarius cedriolens 274 4e-73 96.43
/cortinarius8	159	AF389154 Cortinarius bulliardii 234 1e-61 149/158 (94%)	UDB002195 Cortinarius colymbadinus 272 1e-72 97.48
/cortinarius9	138	AJ236069 Cortinarius porphyropus 410 e-114 216/219 (98%)	UDB001172 Cortinarius porphyropus 388 2e-107 98.63
/hygrophorus4	12827	DQ490631 Hygrophorus pudorinus 62 2e-09 46/51 (90%)	FJ845411 Hygrophorus piceae 307 5e-83 95.38

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
/inocybe3	2303	AY751558 cf Inocybe sp 333 4e-91 173/175 (98%)	HQ604245 Inocybe nitidiuscula 324 4e-88 100.00
/inocybe4	254	AM882750 Astrosporina alpigenes 196 3e-50 147/159 (92%)	HQ604245 Inocybe nitidiuscula 303 6e-82 97.74
/inocybe5	172	AM882787 Inocybe leptophylla 408 e-114 230/234 (98%)	FJ554127 uncultured Boletales 424 6e-118 99.57
/laccaria1	471	DQ149854 Laccaria nobilis 373 e-103 191/192 (99%)	FJ845417 Laccaria bicolor 355 2e-97 100.00
/laccaria2	111	DQ179121 Laccaria bicolor 234 1e-61 121/122 (99%)	FJ845417 Laccaria bicolor 226 8e-59 100.00
/meliniomyces1	549	EF517302 Meliniomyces bicolor 224 1e-58 129/133 (96%)	FJ827193 uncultured Helotiales 281 2e-75 98.15
/meliniomyces2	238	EF093180 Meliniomyces bicolor 240 3e-63 164/175 (93%)	FJ827193 uncultured Helotiales 303 6e-82 97.74
/meliniomyces3	105	AM084704 Rhizoscyphus ericae 254 2e-67 157/164 (95%)	HQ157928 Meliniomyces vraolstadiae 289 1e-77 98.77
/meliniomyces4	104	M084704 Rhizoscyphus ericae 254 2e-67 157/164 (95%)	HQ157928 Meliniomyces vraolstadiae 289 1e-77 98.77
/piloderma7	5121	DQ469288 Piloderma lanatum 153 4e-37 92/97 (94%)	FJ236851 uncultured Piloderma 287 5e-77 99.37
/piloderma8	2873	DQ469288 Piloderma lanatum 139 8e-33 92/98 (93%)	FJ236851 uncultured Piloderma 353 6e-97 100.00
/piloderma9	4250	DQ469288 Piloderma lanatum 105 6e-23 93/104 (89%)	HQ271370 uncultured Piloderma 180 6e-45 94.87
/piloderma10	2779	AJ438982 Piloderma croceum 157 3e-38 88/91 (96%)	FJ236851 uncultured Piloderma 235 2e-61 93.71
/piloderma11	333	DQ469288 Piloderma lanatum 105 6e-23 93/104 (89%)	HQ271370 uncultured Piloderma 167 4e-41 95.33
/piloderma12	244	DQ469288 Piloderma lanatum 161 2e-39 118/129 (91%)	HQ271370 uncultured Piloderma 289 1e-77 100.00

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
/pseudotomentella 1	2215	AF274768 Pseudotomentella mucidula 210 2e-54 109/110 (99%)	UDB001617 Pseudotomentella griseoper. 239 1e-62 95.92
/pseudotomentella 3	4462	EU846255 Polyozellus multiplex 198 9e-51 140/152 (92%)	UDB001617 Pseudotomentella griseoper. 195 3e-49 87.15
/pseudotomentella 4	225	AM490945 Pseudotomentella humicola 216 2e-56 112/113 (99%)	UDB000277 Pseudotomentella humicola 137 3e-32 98.70
/pseudotomentella 5	212	AF274768 Pseudotomentella mucidula 182 6e-46 134/147 (91%)	UDB001617 Pseudotomentella griseoper. 285 2e-76 94.62
/pseudotomentella 6	310	AJ889968 Pseudotomentella tristis 168 7e-42 139/157 (88%)	UDB001617 Pseudotomentella griseoper. 228 3e-59 92.95
/russula-lactarius1	284	AY061657 Russula aquosa 119 4e-27 63/64 (98%)	UDB000914 Russula nana 161 2e-39 90.98
/russula-lactarius3	1071	AY061733 Russula nauseosa 214 9e-56 115/116 (99%)	UDB001641 Russula versicolor 189 1e-47 95.80
/russula-lactarius4	120	AF418621 Russula raoultii 266 5e-71 187/203 (92%)	UDB000914 Russula nana 350 8e-96 98.03
/russula-lactarius5	3796	AY061668 Russula curtipes 289 3e-78 178/186 (95%)	UDB002484 Russula aurantioflammans 298 3e-80 95.70
/russula-lactarius6	818	AF093456 Lactarius deliciosus 212 3e-55 107/107 (100%)	UDB000866 Lactarius deterrimus 176 7e-44 96.30
/russula-lactarius7	546	EU248592 Russula queletii 371 e-102 187/187 (100%)	UDB000316 Russula queletii 265 3e-70 92.43
/russula-lactarius8	201	AF418612 Russula aeruginea 325 7e-89 197/207 (95%)	UDB001621 Russula aeruginea 326 1e-88 95.19
/sebacina1	160	DQ661898 uncultured fungus 206 3e-53 110/112 (98%)	HQ211919 uncultured Sebacina 202 1e-51 99.11
/sebacina2	108	AM260864 uncultured fungus 224 3e-58 175/193 (90%)	GQ907110 uncultured Sebacina 300 8e-81 94.85
/tomentella-thelephora2	1155	EU819522 Tomentella badia 224 1e-58 130/135 (96%)	FN669284 Thelephoraceae sp B310 235 2e-61 97.14

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
/tomentella-thelephora3	109	EU819522 Tomentella badia 180 1e-45 102/105 (97%)	FN669284 Thelephoraceae sp B310 193 7e-49 99.07
/wilcoxina1	1377	DQ069000 Wilcoxina mikolae 226 4e-59 166/182 (91%)	DQ200834 Trichophaea cf hybrida KH0439 348 3e-95 100.00
Agaricales1	511	EU292586 uncultured fungus 196 3e-50 111/114 (97%)	HQ212056 uncultured Ramariopsis 193 7e-49 97.37
Agaricales2	177	DQ490638 Kuehneromyces rostratus 182 3e-46 101/104 (97%)	FJ596765 Pholiota sp TENN61700 187 3e-47 99.04
Agaricales3	162	AY228353 Clavaria acuta 96 7e-20 103/118 (87%)	HQ212158 uncultured Clavaria 230 7e-60 98.47
Boletales1	579	AM747522 Coniophora olivacea 402 e-112 203/203 (100%)	GU187519 Coniophora prasinoides 375 1e-103 100.00
Chaetothyriales1	170	EU035403 Cladophialophora chaetospira 143 4e-34 131/148 (88%)	FJ265750 Cladophialophora sp L359 211 3e-54 88.70
Helotiales1	247	AF486132 Phialocephala virens 157 3e-38 154/174 (88%)	HQ021923 uncultured Helotiales 313 9e-85 100.00
Helotiales2	562	AF169309 Hymenoscyphus monotropae 151 2e-36 150/168 (89%)	HQ212138 uncultured Helotiales 294 3e-79 98.79
Helotiales4	103	AF011327 Cadophora finlandica 131 2e-30 103/113 (91%)	HQ157913 Helotiaceae sp VI GK 2010 340 5e-93 99.47
Helotiales5	576	AF486132 Phialocephala virens 214 1e-55 149/160 (93%)	HM488471 uncultured Helotiales 276 1e-73 98.10
Helotiales8	154	EF093148 Helotiales sp 289 4e-78 156/158 (98%)	FR667221 Chalara sp CCF 3976 279 8e-75 98.73
Helotiales9	564	U57495 Hyaloscypha aureliella 236 3e-62 147/155 (94%)	FJ475650 uncultured Helotiales 248 2e-65 95.51
Helotiales10	535	DQ309193 uncultured fungus 258 1e-68 144/146 (98%)	HM146841 uncultured Helotiales 252 2e-66 97.95
Helotiales11	411	EF029237 Helicodendron luteoalbum 228 7e-60 131/135 (97%)	EF029238 Helicodendron luteoalbum 226 9e-59 97.04

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
Helotiales12	368	AY805569 Leptodontidium elatius 289 3e-78 155/158 (98%)	UDB003048 Trichopezizella relicina 265 2e-70 95.73
Helotiales13	109	DQ437689 uncultured ectomycorrhizal fungus 323 3e-88 166/167 (99%)	FJ827188 uncultured Helotiales 272 1e-72 96.36
Helotiales14	364	DQ093752 Chalara microchona 289 3e-78 158/161 (98%)	AM181407 uncultured Helotiales 267 7e-71 96.89
Helotiales15	170	EU040232 Hyalodendriella betulae 248 1e-65 167/177 (94%)	FR667230 Chalara piceae abietis 318 2e-86 99.43
Helotiales16	169	FJ000377 Articulospora tetracladia 268 1e-71 158/163 (96%)	FJ827196 uncultured Helotiales 276 1e-73 97.53
Helotiales17	167	EF434152 uncultured fungus 278 2e-74 203/220 (92%)	FJ554025 uncultured Helotiales 337 7e-92 94.55
Helotiales18	146	EU882733 Phialocephala fortinii 327 1e-89 165/165 (100%)	EU882733 Phialocephala fortinii 305 1e-82 100.00
Helotiales19	111	AF486132 Phialocephala virens 226 4e-59 159/170 (93%)	HM488471 uncultured Helotiales 287 5e-77 97.62
Helotiales20	108	EF029197 Helicodendron websteri 260 3e-69 157/163 (96%)	AY465463 Phialophora sp GS10N3a 300 6e-81 100.00 0
Helotiales21	105	AF486132 Phialocephala virens 188 8e-48 156/171 (91%)	EF619698 uncultured Helotiales 281 2e-75 97.02
Leotiomycetes1	176	EU113188 uncultured fungus 246 6e-65 145/152 (95%)	HQ211516 uncultured Leotiomycetes 303 5e-82 99.40
Leotiomycetes3	330	EU035414 Cylindrosympodium lauri 56 7e-08 37/40 (92%)	HM488475 uncultured Leotiomycetes 117 6e-26 85.96
Leotiomycetes4	536	EU678392 Leohumicola levissima 297 1e-80 156/158 (98%)	AY706329 Leohumicola minima 281 2e-75 98.73
mitosporic Ascomycota1	274	AY729937 Gyoerffyella rotula 278 1e-74 152/156 (97%)	GU998652 uncultured Helotiales 289 1e-77 100.00
mitosporic Ascomycota2	107	FJ000372 Tetracladium palmatum 260 2e-69 155/161 (96%)	FJ000374 Tetracladium setigerum 265 2e-70 96.88

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
Mortierellales1	6546	AJ878779 Mortierella horticola 98 2e-20 52/53 (98%)	HQ022258 uncultured Mortierella 313 9e-85 100.00
Mortierellales2	3824	DQ093723 Mortierella gamsii 184 1e-46 141/153 (92%)	FN565381 uncultured zygomycete 272 1e-72 99.34
Mortierellales3	1668	AJ878780 Mortierella hyalina 178 6e-45 138/152 (90%)	EU806601 uncultured soil fungus 244 3e-64 97.24
Mortierellales5	437	AJ878782 Mortierella macrocystis 280 3e-75 154/157 (98%)	EU807054 uncultured soil fungus 283 6e-76 99.36
Mortierellales6	2230	AJ878778 Mortierella humilis 276 4e-74 153/155 (98%)	JF439486 Mortierella humilis 274 4e-73 98.71
Mortierellales7	413	DQ093725 Mortierella sp 339 6e-93 174/175 (99%)	DQ093725 Mortierella sp aurim1236 318 2e-86 99.43
Mortierellales8	274	FJ025143 Mortierella alpina 60 4e-09 39/42 (92%)	HQ212224 uncultured Mortierella 235 2e-61 95.89
Mortierellales9	284	AJ878780 Mortierella hyalina 141 1e-33 116/129 (89%)	EU806601 uncultured soil fungus 224 3e-58 95.17
Mortierellales10	139	AJ878778 Mortierella humilis 86 7e-17 49/51 (96%)	HQ873375 uncultured fungus 270 5e-72 99.33
Mortierellales11	122	FJ025143 Mortierella alpina 82 1e-15 44/45 (97%)	FN678837 Mortierellales sp GF5V1a 137 5e-32 83.03
Onygenales2	338	AY354254 Oidiodendron scytaloides 262 7e-70 153/160 (95%)	AF062789 Oidiodendron chlamydosporicum 259 1e-68 95.15
Pezizomycotina1	341	DQ497980 uncultured Pezizomycotina 210 3e-54 128/134 (95%)	FJ554130 uncultured Pezizomycotina 219 1e-56 96.27
Pezizomycotina2	165	EU529971 uncultured ectomycorrhizal fungus 317 2e-86 163/164 (99%)	HQ211950 uncultured Pezizomycotina 298 2e-80 99.39
Pezizomycotina3	129	EF029203 Helicoon fuscosporum 285 4e-77 157/160 (98%)	EU035472 Venturia sp CBS 68174 272 1e-72 97.50
Tremellales2	947	AF444350 Cryptococcus terricola 315 5e-86 162/163 (99%)	FN298664 Cryptococcus terricola 296 8e-80 99.39

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
unknown2	248	DQ309201 uncultured fungus 204 2e-52 118/123 (95%)	HQ215901 uncultured fungus 228 2e-59 100.00
unknown4	876	DQ309123 uncultured fungus 34 0.30 26/29 (89%)	GU174299 uncultured fungus 178 2e-44 94.07
unknown8	5949	DQ233843 uncultured ectomycorrhizal fungus 204 1e-52 109/111 (98%)	HM164554 uncultured fungus 195 2e-49 98.20
unknown9	160	EF521261 uncultured fungus 137 3e-32 91/97 (93%)	HQ126589 uncultured fungus 202 1e-51 97.48
unknown10	414	FJ152543 uncultured ectomycorrhiza Atheliaceae 198 9e-51 112/116 (96%)	FJ152543 uncultured Pezizomycotina 193 8e-49 96.55
unknown15	1098	EF434026 uncultured fungus 48 2e-05 42/48 (87%)	GQ160038 uncultured fungus 180 5e-45 96.36
unknown16	885	EU292658 uncultured fungus 129 6e-30 86/93 (92%)	FN295087 uncultured fungus 191 3e-48 97.32
unknown17	679	AF481369 ectomycorrhizal root tip 81sepB 210 2e-54 113/114 (99%)	FM992983 uncultured ectomycorrhizal .. 207 3e-53 97.56
unknown18	723	EU292623 uncultured fungus 192 7e-49 115/121 (95%)	GQ160022 uncultured fungus 204 4e-52 93.01
unknown19	2433	EU292658 uncultured fungus 121 1e-27 86/93 (92%)	GU174348 uncultured fungus 171 3e-42 97.03
unknown20	455	DQ182440 uncultured fungus 38 0.016 29/31 (93%)	NO HITS FOUND.
unknown21	936	EF434064 uncultured fungus 182 5e-46 110/116 (94%)	FN294751 uncultured fungus 215 2e-55 100.00
unknown22	631	EU292623 uncultured fungus 194 1e-49 110/114 (96%)	GQ160032 uncultured fungus 206 9e-53 99.12
unknown23	458	EU292658 uncultured fungus 131 2e-30 90/98 (91%)	FN295085 uncultured fungus 189 9e-48 98.17
unknown24	225	EF434080 uncultured fungus 76 9e-14 74/86 (86%)	NO HITS FOUND.

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
unknown25	218	DQ421137 uncultured soil fungus 96 2e-19 70/75 (93%)	DQ421136 uncultured soil fungus 176 1e-43 83.49
unknown26	143	U66437 Rickenella pseudogrisella 40 0.007 23/24 (95%)	NO HITS FOUND.
unknown27	273	EF434113 uncultured fungus 264 2e-70 136/137 (99%)	EF434113 uncultured fungus 248 2e-65 99.27
unknown28	134	EU622343 Hydnellum ferrugineum 30 5.0 15/15 (100%)	NO HITS FOUND.
unknown29	128	EF434108 uncultured fungus 121 1e-27 86/93 (92%)	GU174348 uncultured fungus 176 7e-44 98.02
unknown30	121	AF504878 uncultured fungus 80 5e-15 59/64 (92%)	DQ421207 uncultured soil fungus 100 5e-21 85.86
unknown31	120	EF434064 uncultured fungus 82 1e-15 99/117 (84%)	GQ160171 uncultured fungus 219 1e-56 100.00
unknown32	118	EU292657 uncultured fungus 34 0.32 17/17 (100%)	NO HITS FOUND.
unknown33	114	EF434064 uncultured fungus 168 8e-42 106/113 (93%)	GQ160163 uncultured fungus 209 7e-54 100.00
unknown34	113	DQ421288 uncultured soil fungus 60 9e-09 45/50 (90%)	HQ125769 uncultured fungus 278 4e-74 94.09
unknown35	112	EU517068 Tremellales sp 32 1.1 16/16 (100%)	NO HITS FOUND.
unknown36	106	FJ000372 Tetracladium palmatum 260 2e-69 155/161 (96%)	NO HITS FOUND.
unknown37	101	DQ497970 uncultured ectomycorrhiza Atheliaceae 42 0.001 30/33 (90%)	NO HITS FOUND.
unknown38	101	EF434129 uncultured fungus 281 1e-75 160/166 (96%)	GU083180 uncultured soil fungus 279 9e-75 96.99
unknown Ascomycota2	486	AY970069 uncultured ascomycete 291 1e-78 184/195 (94%)	HQ022099 uncultured Verrucariales 298 3e-80 94.36

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
unknown Ascomycota4	847	EU554708 uncultured fungus 52 2e-06 54/62 (87%)	FN294780 uncultured Ascomycota 257 4e-68 98.00
unknown Ascomycota5	252	EF016385 Cladophialophora minutissima 82 1e-15 66/73 (90%)	HQ022171 uncultured Verrucariales 272 2e-72 94.92
unknown Ascomycota6	744	EU292664 uncultured fungus 155 1e-37 97/102 (95%)	FJ552850 uncultured Schizosaccharomyces 206 1e-52 98.29
unknown Ascomycota7	149	AY266155 Mycocentrospora acerina 248 8e-66 139/141 (98%)	HM107423 Thyrostroma carpophilum 237 4e-62 97.16
unknown Ascomycota8	133	EU292664 uncultured fungus 121 2e-27 83/89 (93%)	FJ552850 uncultured Schizosaccharomyces 200 4e-51 99.11
unknown Ascomycota9	131	EU807127 uncultured soil fungus 204 2e-52 135/147 (91%)	HQ022301 uncultured Verrucariales 222 1e-57 94.48
unknown Ascomycota10	120	DQ529303 Pseudeurotium bakeri 129 6e-30 116/133 (87%)	GU998429 uncultured Ascomycota 233 7e-61 92.26
unknown Basidiomycota4	173	EU292658 uncultured fungus 176 3e-44 101/105 (96%)	HQ212086 uncultured Basidiomycota 172 9e-43 96.19

c)

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
/amphinema-tylospora1	5282	AF052556 Tylospora asterophora 238 1e-62 157/164 (95%)	GU550116 Tylospora asterophora 272 2e-72 93.16
/amphinema-tylospora2	112	AY010283 Tylospora fibrillosa 176 2e-44 89/89 (100%)	HM581907 Tylospora fibrillosa 171 3e-42 97.06
/amphinema-tylospora3	2122	AY010283 Tylospora fibrillosa 248 8e-66 125/125 (100%)	AY010283 Tylospora fibrillosa 231 2e-60 100.00
/amphinema-tylospora4	952	AF052562 Tylospora fibrillosa 224 1e-58 122/125 (97%)	AF052562 Tylospora fibrillosa 215 2e-55 97.60
/amphinema-tylospora5	163	AF052562 Tylospora fibrillosa 198 5e-51 109/112 (97%)	DQ482029 uncultured Tylospora 207 3e-53 100.00

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
/amphinema-tylospora6	640	AY010283 Tylospora fibrillosa 250 2e-66 133/134 (99%)	AY010283 Tylospora fibrillosa 246 8e-65 98.57
/amphinema-tylospora7	424	AY219839 Amphinema byssoides 262 5e-70 135/136 (99%)	FJ554364 uncultured Amphinema 252 1e-66 100.00
/amphinema-tylospora8	3167	AF052556 Tylospora asterophora 178 5e-45 110/114 (96%)	GU550116 Tylospora asterophora 187 4e-47 95.76
/amphinema-tylospora12	1538	AF052556 Tylospora asterophora 285 5e-77 176/184 (95%)	GU550116 Tylospora asterophora 294 4e-79 95.21
/amphinema-tylospora13	322	EF493272 Amphinema byssoides 94 3e-19 61/65 (93%)	FM992887 uncultured Amphinema 257 3e-68 99.30
/amphinema-tylospora14	501	EF493272 Amphinema byssoides 94 3e-19 61/65 (93%)	FM992887 uncultured Amphinema 246 7e-65 99.27
/amphinema-tylospora18	1645	AY219839 Amphinema byssoides 172 4e-43 135/148 (91%)	UDB001719 Amphinema 268 2e-71 99.32
/amphinema-tylospora19	467	AY219839 Amphinema byssoides 119 7e-27 121/141 (85%)	GQ162811 Amphinema diadema 305 2e-82 95.81
/amphinema-tylospora20	121	AF052556 Tylospora asterophora 135 6e-32 97/104 (93%)	GU550116 Tylospora asterophora 152 1e-36 92.59
/cortinarius10	1117	DQ517404 Cortinarius duracinus 321 8e-88 169/170 (99%)	GQ159884 Cortinarius velenovskyi 307 4e-83 99.41
/cortinarius11	789	EU837212 Cortinarius barlowensis 406 e-113 212/213 (99%)	UDB002218 Cortinarius anomalus 351 2e-96 96.28
/hygrophorus5	2455	DQ097873 Hygrophorus albicastaneus 420 e-117 254/265 (95%)	FJ596880 Camarophyllus pratensis 453 8e-127 96.39
/inocybe3	2162	AM882751 Astrosporina alpigenes 278 1e-74 162/168 (96%)	HQ604245 Inocybe nitidiuscula 375 1e-103 99.52
/inocybe4	119	AM882751 Astrosporina alpigenes 309 4e-84 181/188 (96%)	HQ604245 Inocybe nitidiuscula 407 5e-113 99.12
/inocybe6	712	DQ367905 Inocybe lanuginosa var 444 e-124 234/236 (99%)	UDB002391 Inocybe lanuginosa 429 1e-119 99.58

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
/inocybe7	117	AM882913 Inocybe nitidiuscula 341 1e-93 198/204 (97%)	HQ604087 Inocybe cf pruinosa UBC F18459 364 3e-100 99.02
/inocybe8	105	AM882888 Inocybe fuscidula 176 4e-44 170/191 (89%)	HQ604368 Inocybe sindonia 340 5e-93 96.19
/laccaria3	222	DQ149854 Laccaria nobilis 450 e-126 237/239 (99%)	FJ845417 Laccaria bicolor 435 3e-121 99.58
/meliniomyces2	111	AF011327 Cadophora finlandica 206 3e-53 123/128 (96%)	HQ157926 Meliniomyces bicolor 241 4e-63 93.87
/meliniomyces5	638	AF011327 Cadophora finlandica 105 1e-22 97/109 (88%)	HQ211624 uncultured Helotiales 300 8e-81 95.72
/piloderma5	148	DQ469288 Piloderma lanatum 299 3e-81 174/179 (97%)	UDB001733 Piloderma 327 3e-89 100.00
/piloderma13	132	DQ365673 Piloderma fallax 101 1e-21 121/142 (85%)	GQ159951 uncultured fungus 255 1e-67 99.30
/pseudotomentella 1	947	AF274768 Pseudotomentella mucidula 232 6e-61 127/129 (98%)	UDB001617 Pseudotomentella griseoper. 254 5e-67 94.55
/pseudotomentella 2	192	AF274768 Pseudotomentella mucidula 301 1e-81 183/192 (95%)	UDB001617 Pseudotomentella griseoper. 311 4e-84 91.74
/pseudotomentella 7	584	AJ889968 Pseudotomentella tristis 216 5e-56 189/214 (88%)	UDB003209 Pseudotomentella 267 9e-71 88.55
/russula-lactarius3	173	AY061733 Russula nauseosa 301 9e-82 183/188 (97%)	HQ604852 Russula puellaris 327 3e-89 98.40
/russula-lactarius5	11931	AY061668 Russula curtipes 305 6e-83 186/194 (95%)	EU248593 Russula aff curtipes UC 1859959 322 2e-87 96.89
/russula-lactarius8	1801	AF418612 Russula aeruginea 333 3e-91 210/222 (94%)	UDB001621 Russula aeruginea 340 5e-93 94.62
/russula-lactarius9	303	AY061685 Russula laricina 274 2e-73 147/150 (98%)	UDB001716 Russula nauseosa 261 3e-69 98.00
/russula-lactarius10	1300	AF418631 Russula firmula 321 9e-88 182/186 (97%)	UDB000359 Russula firmula 320 6e-87 97.85

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
/russula-lactarius11	103	AF418616 Russula fellea 153 3e-37 92/96 (95%)	UDB000914 Russula nana 193 8e-49 95.90
/sebacina3	102	DQ309149 uncultured fungus 222 1e-57 174/190 (91%)	GQ907148 uncultured Sebacina 331 3e-90 98.41
/sistotrema1	145	DQ397337 Sistotrema coronilla 127 2e-29 94/102 (92%)	EF521234 uncultured fungus 219 1e-56 99.17
/tomentella-thelephora4	120	EU819522 Tomentella badia 309 4e-84 189/198 (95%)	EU819522 Tomentella badia 316 8e-86 95.48
/tuber-helvella1	181	AY558743 Barssia oregonensis 303 2e-82 153/153 (100%)	AY558743 Barssia oregonensis 283 6e-76 100.00
/wilcoxina1	271	U38563 Wilcoxina mikolae 135 6e-32 89/96 (92%)	DQ069051 Wilcoxina sp aurim735 185 1e-46 98.11
/wilcoxina2	783	U38563 Wilcoxina mikolae 127 1e-29 88/96 (91%)	DQ200834 Trichophaea cf hybrida KH0439 172 8e-43 97.06
Agaricales4	529	EU118617 Clavulinopsis helvola 117 1e-26 65/67 (97%)	UDB001534 Clavulinopsis helvola 145 2e-34 93.81
Agaricales5	415	FM208862 Hygrocybe conica var 200 2e-51 177/198 (89%)	FM208878 Hygrocybe conica var conica 248 3e-65 90.00
Agaricales6	319	EU118617 Clavulinopsis helvola 119 3e-27 66/68 (97%)	UDB001534 Clavulinopsis helvola 147 5e-35 93.88
Agaricales7	355	DQ182453 uncultured Agaricales 198 1e-50 169/188 (89%)	FJ553835 uncultured Agaricomycetes 315 3e-85 97.31
Agaricales8	144	AY228353 Clavaria acuta 212 3e-55 110/111 (99%)	FJ554393 uncultured Agaricomycetes 206 9e-53 100.00
Agaricales9	103	DQ494680 Nolanea strictia 113 2e-25 100/116 (86%)	EU669311 Nolanea cf verna OSC 66363 196 5e-50 100.00
Auriculariales1	294	DQ873660 Protodontia piceicola 230 3e-60 141/148 (95%)	DQ873660 Protodontia piceicola 241 4e-63 94.38
Eurotiales1	110	AF033489 Penicillium kojigenum 337 1e-92 170/170 (100%)	GU565124 Penicillium sp GZU BCECDJL3 1 315 2e-85 100.00

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
Helotiales2	358	DQ914672 Cystodendron sp 192 5e-49 110/113 (97%)	HQ212138 uncultured Helotiales 204 3e-52 99.12
Helotiales3	187	AY129286 Pseudeurotium zonatum 178 7e-45 135/150 (90%)	HQ845751 Helotiales sp PIMO 265 250 6e-66 94.97
Helotiales5	373	AF486132 Phialocephala virens 242 6e-64 163/174 (93%)	HM488471 uncultured Helotiales 302 2e-81 98.26
Helotiales7	102	AF011327 Cadophora finlandica 117 3e-26 97/107 (90%)	HQ157918 Helotiaceae sp III GK 2010 340 4e-93 100.00
Helotiales12	124	DQ437689 uncultured ectomycorrhizal fungus 315 8e-86 166/167 (99%)	FJ827188 uncultured Helotiales 265 2e-70 95.78
Helotiales13	127	DQ093752 Chalara microchona 339 4e-93 174/175 (99%)	DQ093752 Chalara microchona 318 2e-86 99.43
Helotiales16	108	DQ309243 uncultured fungus 244 2e-64 145/151 (96%)	FJ475761 uncultured Helotiales 244 3e-64 96.03
Helotiales19	104	EF029197 Helicodendron websteri 236 4e-62 152/159 (95%)	HQ599581 Xenopolyscytalum pinea 285 2e-76 99.37
Helotiales20	264	AF486132 Phialocephala virens 153 3e-37 121/133 (90%)	EF619698 uncultured Helotiales 220 4e-57 96.95
Helotiales21	208	U57495 Hyaloscypha aureliella 256 4e-68 148/153 (96%)	HQ157879 Helotiaceae sp VII GK 2010 281 2e-75 100.00
Helotiales22	162	DQ273335 uncultured Pezizomycotina 232 8e-61 133/137 (97%)	FJ440903 uncultured Helotiales 237 4e-62 96.53
Helotiales23	116	DQ529303 Pseudeurotium bakeri 147 2e-35 104/114 (91%)	HQ845751 Helotiales sp PIMO 265 185 1e-46 94.21
Helotiales24	112	U57495 Hyaloscypha aureliella 230 2e-60 148/156 (94%)	U57495 Hyaloscypha aureliella 243 1e-63 94.87
Helotiales25	111	AF486132 Phialocephala virens 186 3e-47 153/170 (90%)	DQ914733 Helotiales sp EXP0568F 252 2e-66 94.05
Leotiomycetes1	128	AY627804 Epacris root associated fungus 285 8e-77 162/168 (96%)	HQ211516 uncultured Leotiomycetes 335 2e-91 98.94

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
Leotiomycetes3	245	EU449953 Sympodiella acicola 42 0.001 30/33 (90%)	NO HITS FOUND.
Leotiomycetes5	217	AJ133432 Calyptrozyma arxii 131 1e-30 108/122 (88%)	GQ154586 Collophora paarla 180 7e-45 93.44
Leotiomycetes6	182	AJ133432 Calyptrozyma arxii 92 1e-18 62/66 (93%)	FJ553604 uncultured Pezizomycotina 187 4e-47 93.65
Leotiomycetes7	146	DQ195779 Fulvoflamma eucalypti 163 4e-40 140/156 (89%)	FR846479 Leotiomycetes sp NK266 172 1e-42 97.06
Leotiomycetes8	114	EU678379 Leohumicola verrucosa 115 6e-26 68/70 (97%)	EU940163 Arachnopeziza variepilosa 193 7e-49 99.07
Leotiomycetes9	106	AJ133432 Calyptrozyma arxii 98 2e-20 88/97 (90%)	AY371513 Cadophora sp NH1 2 217 7e-56 90.85
Leucosporidiales1	104	AF444630 Mastigobasidium intermedium 94 5e-19 56/59 (94%)	FR719968 Mastigobasidium intermedium 163 8e-40 87.10
mitosporic Ascomycota1	281	AY729937 Gyoerffyella rotula 278 1e-74 152/156 (97%)	GU998652 uncultured Helotiales 289 1e-77 100.00
mitosporic Ascomycota3	354	FJ000363 Tetracladium setigerum 278 9e-75 140/140 (100%)	FJ000372 Tetracladium palmatum 259 9e-69 100.00
mitosporic Ascomycota4	137	AY706334 Humicola grisea var 242 5e-64 138/142 (97%)	HQ115678 Trichocladium asperum 244 3e-64 97.87
Mortierellales1	9680	AJ878779 Mortierella horticola 98 2e-20 52/53 (98%)	HQ022258 uncultured Mortierella 250 6e-66 98.59
Mortierellales2	1495	DQ093723 Mortierella gamsii 159 6e-39 128/140 (91%)	FN565381 uncultured zygomycete 248 2e-65 99.28
Mortierellales3	755	AJ878780 Mortierella hyalina 180 1e-45 129/141 (91%)	EU806603 uncultured soil fungus 235 1e-61 98.51
Mortierellales6	1453	AJ878778 Mortierella humilis 295 5e-80 170/173 (98%)	JF439486 Mortierella humilis 300 7e-81 98.27
Mortierellales7	103	AJ541799 Mortierella sp 309 5e-84 166/168 (98%)	AJ541799 Mortierella sp Finse 15 07 00 298 2e-80 98.81

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
Mortierellales8	306	FJ025182 Mortierella alpina 66 9e-11 39/41 (95%)	HQ212224 uncultured Mortierella 326 1e-88 98.40
Mortierellales11	212	EF519906 Mortierella alpina 78 2e-14 42/43 (97%)	GU174322 uncultured fungus 204 5e-52 89.29
Mortierellales12	472	AJ878780 Mortierella hyalina 182 4e-46 137/150 (91%)	EU806601 uncultured soil fungus 246 7e-65 97.90
Mortierellales13	426	AJ878779 Mortierella horticola 80 3e-15 43/44 (97%)	HQ873375 uncultured fungus 198 2e-50 97.46
Mortierellales14	346	DQ093723 Mortierella gamsii 129 5e-30 129/145 (88%)	GU327518 uncultured Mortierella 191 3e-48 91.10
Mortierellales15	123	AJ878504 Mortierella elongata 100 3e-21 87/94 (92%)	HQ446023 uncultured fungus 158 2e-38 95.10
Mortierellales16	232	DQ093723 Mortierella gamsii 143 3e-34 108/116 (93%)	FJ161929 Mortierella exigua 172 1e-42 93.97
Mortierellales17	117	AJ878504 Mortierella elongata 204 1e-52 139/147 (94%)	JF519043 uncultured Mortierella 230 8e-60 95.24
Mortierellales18	111	EF519900 Mortierella alpina 210 1e-54 106/106 (100%)	FJ861398 Mortierella alpina 196 5e-50 100.00
Onygenales2	269	AY354254 Oidiodendron scytaloides 260 3e-69 152/159 (95%)	AF062789 Oidiodendron chlamydosporicum 257 4e-68 95.12
Pezizomycotina2	218	EU482220 Cryptosporiopsis actinidiae 84 3e-16 58/62 (93%)	HQ211994 uncultured Pezizomycotina 279 8e-75 100.00
Pezizomycotina4	108	AF178563 Porosphaerella cordanophora 216 3e-56 150/161 (93%)	JF340260 Spadicoides bina 235 2e-61 93.21
Rhytismatales1	105	EU652347 Cudonia monticola 268 1e-71 161/166 (96%)	EU652347 Cudonia monticola 281 3e-75 96.53
Tremellales1	794	AF444350 Cryptococcus terricola 238 7e-63 120/120 (100%)	FN298664 Cryptococcus terricola 222 1e-57 100.00
Tremellales2	965	AF444350 Cryptococcus terricola 204 9e-53 117/119 (98%)	FN298664 Cryptococcus terricola 207 3e-53 98.32

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
Tremellales3	302	AF444350 Cryptococcus terricola 311 8e-85 164/165 (99%)	FN298664 Cryptococcus terricola 298 2e-80 99.39
unknown2	223	DQ309201 uncultured fungus 200 2e-51 116/121 (95%)	HQ215901 uncultured fungus 224 3e-58 100.00
unknown4	2386	DQ309123 uncultured fungus 34 0.30 26/29 (89%)	GU174299 uncultured fungus 178 2e-44 94.07
unknown6	979	EU292602 uncultured fungus 379 e-105 204/209 (97%)	HQ124488 uncultured fungus 375 1e-103 99.04
unknown8	653	DQ233843 uncultured ecm fungus 202 5e-52 108/110 (98%)	HM164554 uncultured fungus 193 7e-49 98.18
unknown9	117	EF521261 uncultured fungus 131 2e-30 88/94 (93%)	HQ126589 uncultured fungus 196 6e-50 97.41
unknown15	753	EF434026 uncultured fungus 40 0.005 41/48 (85%)	GQ160038 uncultured fungus 209 7e-54 100.00
unknown22	118	EU292623 uncultured fungus 198 9e-51 115/120 (95%)	GQ160022 uncultured fungus 211 2e-54 98.33
unknown23	291	EU292658 uncultured fungus 139 6e-33 91/98 (92%)	FN295085 uncultured fungus 187 3e-47 99.05
unknown27	122	EF434113 uncultured fungus 232 7e-61 129/133 (96%)	HQ124795 uncultured fungus 233 5e-61 97.12
unknown39	3524	AY702729 uncultured fungus from ecm root 472 e-133 244/246 (99%)	GU083061 uncultured soil fungus 449 1e-125 99.59
unknown40	910	EF434064 uncultured fungus 74 4e-13 115/136 (84%)	GQ160171 uncultured fungus 228 2e-59 97.08
unknown41	662	EU180016 uncultured Soil Clone Group I 68 2e-11 46/49 (93%)	FJ552837 uncultured fungus 196 5e-50 100.00
unknown42	538	EU292658 uncultured fungus 133 5e-31 114/129 (88%)	FN295087 uncultured fungus 220 4e-57 96.30
unknown43	518	AM260905 uncultured fungus 135 1e-31 81/84 (96%)	GQ159992 uncultured fungus 198 1e-50 100.00

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
unknown44	2838	EF434064 uncultured fungus 76 9e-14 104/122 (85%)	GQ160171 uncultured fungus 215 2e-55 98.37
unknown45	385	AM260905 uncultured fungus 131 2e-30 79/82 (96%)	GQ159980 uncultured fungus 198 1e-50 100.00
unknown46	541	EU292600 uncultured fungus 155 1e-37 116/126 (92%)	FN295192 uncultured fungus 207 3e-53 96.77
unknown47	332	EU622343 Hydnellum ferrugineum 30 4.3 15/15 (100%)	NO HITS FOUND.
unknown48	300	EF434064 uncultured fungus 40 0.005 29/32 (90%)	GU174421 uncultured fungus 161 2e-39 90.91
unknown49	224	EU292623 uncultured fungus 238 1e-62 132/136 (97%)	GQ160031 uncultured fungus 252 2e-66 99.29
unknown50	215	DQ396943 Stereocaulon coniophyllum 50 5e-06 28/29 (96%)	NO HITS FOUND.
unknown51	208	EU726309 uncultured Thelephoraceae 38 0.031 19/19 (100%)	HM015729 uncultured fungus 307 4e-83 96.76
unknown52	190	U73493 Peltigera britannica 32 1.1 16/16 (100%)	NO HITS FOUND.
unknown53	180	EU870071 uncultured Cryptococcus 36 0.075 18/18 (100%)	NO HITS FOUND.
unknown54	167	AY509551 Ductifera sucina 52 2e-06 44/50 (88%)	NO HITS FOUND.
unknown55	164	DQ182455 uncultured fungus 38 0.020 22/23 (95%)	GU174419 uncultured fungus 182 2e-45 94.87
unknown56	153	EF592070 uncultured fungus 42 0.002 43/49 (87%)	NO HITS FOUND.
unknown57	139	EU292658 uncultured fungus 129 7e-30 86/93 (92%)	FN295087 uncultured fungus 196 6e-50 97.41
unknown58	136	AY310838 uncultured ectomycorrhizal fungus 291 1e-78 191/202 (94%)	AY310838 uncultured ectomycorrhizal .. 309 1e-83 94.58

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
unknown59	105	EF434113 uncultured fungus 96 1e-19 103/119 (86%)	HQ126528 uncultured fungus 154 5e-37 87.59
unknown60	104	AY970023 uncultured fungus 74 4e-13 108/129 (83%)	NO HITS FOUND.
unknown Ascomycota1	327	EF016385 Cladophialophora minutissima 100 6e-21 53/54 (98%)	HQ022024 uncultured Verrucariales 316 7e-86 98.87
unknown Ascomycota2	369	EU030275 Amorphotheca resinae 123 3e-28 110/126 (87%)	HQ022099 uncultured Verrucariales 211 3e-54 93.66
unknown Ascomycota6	639	EU292634 uncultured fungus 147 3e-35 99/106 (93%)	FJ552850 uncultured Schizosaccharomyces 219 1e-56 99.17
unknown Ascomycota8	104	EU292664 uncultured fungus 107 2e-23 72/78 (92%)	FJ552850 uncultured Schizosaccharomyces 180 5e-45 99.00
unknown Ascomycota11	2199	AY618678 Phialophora verrucosa 143 4e-34 136/153 (88%)	EU940163 Arachnopeziza variepilosa 255 1e-67 97.35
unknown Ascomycota12	526	DQ497970 uncultured ectomycorrhiza Atheliaceae 40 0.005 20/20 (100%)	FJ554360 uncultured Pezizomycotina 206 9e-53 100.00
unknown Ascomycota13	739	AY129287 Pseudeurotium bakeri 107 2e-23 87/98 (88%)	FJ554360 uncultured Pezizomycotina 244 2e-64 99.26
unknown Ascomycota14	307	AY969881 uncultured ascomycete 196 3e-50 102/103 (99%)	AY969881 uncultured Ascomycota 185 1e-46 99.03
unknown Basidiomycota2	1077	EF434153 uncultured fungus 98 2e-20 76/84 (90%)	GQ159934 uncultured fungus 189 8e-48 100.00
unknown Basidiomycota5	4319	DQ481983 uncultured ectomycorrhiza Atheliaceae 161 2e-39 103/109 (94%)	GQ160051 uncultured fungus 174 3e-43 94.69
unknown Basidiomycota6	978	DQ340336 Hyphodontia cineracea 82 3e-15 61/65 (93%)	NO HITS FOUND.
unknown Basidiomycota7	373	EF521256 uncultured fungus 48 2e-05 24/24 (100%)	FJ553628 uncultured Agaricomycetes 185 1e-46 97.27
unknown Basidiomycota8	165	DQ647451 Hyphoderma praetermissum 82 3e-15 44/45 (97%)	GQ411518 Botryobasidium vagum 99.0 3e-20 79.50

Table C.3 cont'd

Final OTU name	# of reads represented	Best NCBI accession number, identity, score, # bases (% match)	Best UNITE accession number, identity, bases, score, and % match
unknown Basidiomycota9	151	AM902055 uncultured basidiomycete 297 2e-80 164/166 (98%)	AM902055 uncultured Basidiomycota 294 3e-79 98.80
unknown Basidiomycota10	117	AM902055 uncultured basidiomycete 196 3e-50 102/103 (99%)	AM902055 uncultured Basidiomycota 185 1e-46 99.03
unknown Glomeromycota1	165	AF133791 uncultured fungus from Acaulospora 98 2e-20 77/85 (90%)	GQ160032 uncultured fungus 87.9 4e-17 83.84

[1]All ectomycorrhizal taxa are identified by lineage (Tedersoo et al., 2010a ECM lifestyle in fungi. Mycorrhiza 20: 217-263. http://unite.ut.ee/EcM_lineages.php), and all other fungal groups are identified to the Family level when possible.

Table C.4 Univariate permutational ANOVA of per-sample presence-absence of a) the mitosporic Ascomycota, and b) the /hygrophorus lineage among control soil, downed, and decayed wood microsites, and among plots.

a)

	Degrees of freedom	SS	MS	F	P(perm)
Plot	2	88444.4	44222.2	132.7	**0.001**
Microsite(Plot)	6	5333.3	888.9	2.67	**0.042**
Residual	36	12000.0	333.3		
Total	44	105777.8			

b)

	Degrees of freedom	SS	MS	F	P(perm)
Plot	2	13777.8	68888.9	3.44	**0.043**
Microsite(Plot)	6	6666.7	1111.1	0.56	0.766
Residual	36	7200.0	2000.0		
Total	44	92444.4.4			

Table C.5 Multivariate permutational ANOVA of per sample presence-absence for the entire community of ECM fungi grouped by lineages among control soil, downed, and decayed wood microsites and among plots.

	Degrees of freedom	SS	MS	F	P(perm)
Plot	2	7634.8	3817.4	6.78	**0.001**
Microsite(Plot)	6	3797.0	632.8	1.12	0.362
Residual	36	20277.6	563.3		
Total	44	31709.4			

Table C.6 Higher-level fungal taxa whose individual occurrence differed significantly among forest plots based on univariate permutational ANOVA.

Lineage or Family	**p-value**
/cenococcum	0.001
/hygrophorus	0.043
/sebacina	0.006
/wilcoxina	0.001
Agaricales	0.001
Cantharellales	0.01
Chaetothyriales	0.001
Eurotiales	0.001
mitosporic Ascomycota	0.001
Pezizales	0.005
Pezizomycotina	0.001
Pleosporales	0.078
unk Basidiomycota	0.001

Table C.7 Multivariate permutational one-way ANOVA of per sample presence-absence for the entire community of ECM species within control soil, downed and decayed wood microsites at a) plot A, b) plot B, and c) plot C.

a)

	Degrees of freedom	SS	MS	F	P(perm)
Microsite	2	2351.8	1175.9	1.12	0.358
Residual	12	12590.3	1049.2		
Total	14	14942.1			

b)

	Degrees of freedom	SS	MS	F	P(perm)
Microsite	2	2578.6	1289.3	1.04	0.459
Residual	12	14907.6	1242.3		
Total	14	17486.2			

c)

	Degrees of freedom	SS	MS	F	P(perm)
Microsite	2	4292.1	2146.1	1.51	0.058
Residual	12	17004.6	1417.1		
Total	14	21296.8			

Table C.8 a) Mean ECM taxon frequency (SD) among microsites across all plots (N=3 plots), and b) ECM taxon frequency (SD) per microsite at individual plots for ECM taxa differing overall and/or at one plot at $p \leq 0.10$. N=5 samples per microsite.

a)

	/am-ty4[1]	/cort2	/melin 1	/pilo10	/am-ty6	/am-ty13	/am-ty20	/lacc3	/melin5	/pseudo 7
	T. fibrillosa	***C. caperatus***	***M. bicolor***	***P. croceum***	***T. fibrillosa***	***A. byssoides***	***T. asterophora***	***L. nobilis***	***C. finlandica***	***P. tristis***
Control soil	0.66 (0.37)	0.07 (0.15)	**0.4 (0.18)**	0.2 (0.18)	0.53 (0.30)	**0.6 (0.15)**	**0.33 (0)**	0	0.07 (0.15)	0
Downed wood	0.66 (0.37)	**0.27 (0.15)**	0.53 (0.18)	0.33 (0)	0.8 (0.45)	0.33 (0)	0.2 (0.18)	**0.27 (0.15)**	0	**0.2 (0.18)**
Decayed wood	0.6 (0.33)	0.07 (0.15)	0.47 (0.18)	**0**	**0.93 (0.15)**	0.47 (0.33)	0.07 (0.15)	0.07 (0.15)	**0.27 (0.15)**	0

b)

	/am-ty4[2]	/cort2[12]	/melin 1[13]	/pilo10[3]	/am-ty6[4]	/am-ty13[4]	/am-ty20[4]	/lacc 3[14]	/melin5[4]	/pseudo 7[14]
	T. fibrillosa	***C. caperatus***	***M. bicolor***	***P. croceum***	***T. fibrillosa***	***A. byssoides***	***T. asterophora***	***L. nobilis***	***C. finlandica***	***P. tristis***
Control soil	1	0.2 (0.45)	0.4 (0.55)	0.6 (0.55)	0	1 (0)	1 (0)	0	0.2 (0.45)	0
Downed wood	0.4 (0.55)	0.8 (0.45)	0.6 (0.55)	1	0.8 (0.45)	0	0.6 (0.55)	0.8 (0.45)	0	0.6 (0.55)
Decayed wood	1	0.2 (0.45)	1 (0)	0	0.8 (0.45)	0.8 (0.45)	0.2 (0.45)	0.2 (0.45)	0.8 (0.45)	0

[1]Not significant at $p \leq 0.1$.
[2]Frequency at plot A only.
[3]Frequency at plot B only.
[4]Frequency at plot C only.