Fungal Community Structure in the Boreal Mixed-Wood Forest

Tonia De Bellis

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Abstract

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Tonia De Bellis, Ph.D.
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Plant communities rely on the belowground microbial community for nutrient uptake, pathogen resistance, growth and establishment. Although the importance of soil microorganisms has been acknowledged for some time, the soil remains one of the least explored habitats on earth. In this thesis I examine the relationships between the aboveground plant community and three functionally different fungal communities in a boreal mixed-wood forest in Québec. The first group, the ectomycorrhizal fungi, are mutualists with many canopy tree species in the plots. As their host is their main carbon source, the communities of these fungi were significantly correlated with the aboveground tree community. These fungi were analyzed using morphological and DNA-based methods, and comparisons of these two techniques showed that each targeted certain groups of ectomycorrhizal fungi. Therefore, the best method to analyze the communities of these fungi would be a combined assessment based on both of these techniques. The second group was the arbuscular mycorrhizal fungi, which form mutualistic associations with the herbaceous plants in the plots. To target these fungi, *Clintonia borealis*, a herbaceous plant found in all the plots was sampled. DNA based identification methods showed that one fungal type was dominant, and was similar to one collected from varied environments and hosts from distant geographical locations. The last group, the saprophytic

microfungi, is not mutualistic with plants but they are decomposers of dead plant material. Statistical analyses showed that the microfungi were most closely correlated with the understory plant species composition than with the soil chemistry or overstory tree species. Significant correlations between plant communities and each of the fungal communities were found, stressing the importance of analyzing both aboveground and belowground components in a combined approach in order to further enhance our understanding of terrestrial ecosystems.

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Contribution of Authors

All 3 chapters of this thesis have been prepared for submission to peer-reviewed journals for publication. In all 3 manuscripts, my supervisor, Dr. P. Widden is a co-author. He has provided valuable guidance in the planning, sampling, lab analysis of all three studies and has edited and provided constructive suggestions for all three manuscripts. Gavin Kernaghan is co-author on 2 of the manuscripts, where he has provided constructive suggestions and has edited earlier versions of the manuscripts. R. Bradley has provided financial support for part of this project and is included as a co-author in the first publication of the thesis.

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General Introduction

Soils contain an immense diversity of microbes, which, to a large extent remains unexplored. One gram of soil may contain up to 10 billion microorganisms of possibly thousands of different species (Torsvik and Ovreas 2000). Fungi are members of the soil microbial community that play a central role in soil ecology and are essential components of healthy forest soils. Fungi are involved in the decomposition of organic matter, disease suppression, plant pathogenicity, and nutrient uptake by plants. They also affect the soil water holding capacity, permeability and are food sources for many other organisms (Christensen 1989). Despite their well-documented role in ecosystem functioning little is known about their community structure and diversity. This is partly due to the fact that only a small fraction of fungi are culturable from the environment; and only an estimated 5% of fungi (Hawksworth 2001) have been classified and named.

Fungal biodiversity is a key component of terrestrial biodiversity. Life belowground and aboveground is interdependent. For example, mycorrhizal fungi interconnect individuals of the same or different species and mycorrhizal fungal diversity plays a role in determining plant biodiversity (van der Heijden et al 1998). Some mycorrhizal fungi induce more positive responses in one plant species than in another; because of these differences the addition of new fungal species leads to increases in survival of a progressively larger number of plant species (van der Heijden et al 1998).

What controls the distribution and abundance of members of the soil fungal community and how these communities change in response to their environment is unclear. Soil structure at any given location is the product of the parent rock, vegetation, and climate (Atlas and Batha 1993). Changes in soil environments resulting from differences in plant cover might be expected to bring about changes in the fungal communities.

In the mixed boreal forest of eastern Canada, disturbances such as fire and outbreaks of spruce budworm (*Choristoneura fumiferana* (Clemens)) create a mosaic-like pattern in the forest characterized by a large variability in species composition of the forest canopy. Aspen (*Populus tremuloides* Michx.), white birch (*Betula papyrifera* Marsh.), and jack pine (*Pinus banksiana* Lamb.) are the three main post fire tree species that gradually get replaced by species such as balsam fir (*Abies balsamea* (L.)) and white spruce (*Picea glauca* (Moench) Voss) (Legaré et al 2001). At the Lake Duparquet Research and Teaching Forest in Abitibi Québec, long term plots with differing proportions of balsam fir (*Abies balsamea* (L.) Mill.), white spruce (*Picea glauca* (Moench) Voss), trembling aspen (*Populus tremuloides* Michx.), and paper birch (*Betula papyrifera* Marsh.) have been established. Taking advantage of the fact that soil chemistry analyses had already been investigated in a previous study (Legaré et al 2001), sampling for this project took place in plots situated on similar clay deposits which had similar chemical properties in order to focus on the relationships between the plant community on the fungal communities.

Ectomycorrhizal Fungi

Mycorrhizae are symbiotic associations between plants and fungi, whereby the fungi provide mineral nutrients to the plant and the fungus acquires carbon compounds from the plant. The roots of most of the dominant tree species in the world's temperate and boreal forests are colonized by ectomycorrhizae (ECM) (Smith and Read 1997). In the ectomycorrhizal association, fungi do not penetrate the cells but fungal hyphae envelop the plant's fine root tips forming a mantle or sheath and grow between the cortical cells, forming a Hartig net. Fungal hyphae extend through the soil, broadening the plant's nutrient depletion zone. The fungi also have the ability to secrete enzymes that allow them to capture nutrients from sources that the plant would otherwise be unable to use (Read 1991). There are approximately 8000 plant species that form ECM. Obligate ectomycorrhizal plant families include the *Betulaceae, Pinaceae, Fagaceae,* and *Dipterocarpaceae* (Dahlberg 2001). Within days of emergence, almost every fine root tip of an ECM plant is colonized. In a boreal forest, 90% of the colonized root tips are located in the top 10 cm of the soil surface (Dahlberg 2001).

Species diversity and species composition of ECMF may have important consequences for growth and nutrient uptake of the host plant. Jonsson et al (2001) showed that birch seedlings inoculated with 8 ECM fungal species had higher growth rates than plants inoculated with only one ECM species, and they also showed that different ECM species varied in their effects on the seedlings. Baxter & Dighton (2001) noted that root biomass increased and shoot biomass

decreased in birch seedlings grown in pots with 4 different ECM species compared to plants grown with a single ECM type.

The factors that influence community development and maintain high species diversity of the ECM fungi are poorly understood. In boreal forests, the richness and complexity of the ECMF communities are in striking contrast to the often species poor stands of trees. There are over 5000 species of ECM fungi belonging to either the Basidiomycota or Ascomycota (Molina et al 1992). Typically the below-ground ECMF community consists of a few common species colonizing 50-70% of available roots and a large number of rare species (Erland and Taylor 2002, Horton and Bruns 1998).

Determining the ECM fungal species diversity in natural ecosystems is a challenging task. Until recently, ECM fungi were assessed by observing aboveground mushroom production. However, many ECM species are hypogeous (form belowground fruiting bodies) and if species do produce aboveground sporocarps, production of these structures is ephemeral and dependent on environmental conditions. Many studies have revealed that mushroom fruiting aboveground is a poor indicator of the types of fungi colonizing tree roots (Gardes and Bruns 1996, Dahlberg et al 1997). Since aboveground fruit body production is a poor indicator of what fungi are colonizing roots, and ECM fungi are not easily grown in culture, the morphotyping technique is a common method used to identify ECMF. Morphotyping involves differentiating the types of fungi present on the root tips based on their macroscopic and microscopic features (Goodman et al 1997). However, the

morphology of the ECM fungi varies with age, host tree species and environmental conditions, and therefore morphotyping is very time consuming and requires a certain level of expertise to identify the fungi even to genus level. Recently, major advances in the study of ECM communities are being made due to the use of molecular tools such as the polymerase chain reaction (PCR). Using fungal specific primers, fungal DNA extracted directly from colonized roots can be amplified and sequences of the PCR products can be used to identify the ECM fungi.

A review of the literature has shown that determinants of the composition of the ECM fungal community include host plant composition and other edaphic factors. Based mainly on sporocarp data, studies have shown that many ECM fungi tend to be specific to a particular host genus (Newton and Haigh 1998, Molina et al 1992). In a greenhouse setting, where different trees were grown in soil from 3 different forests, ECM specificity to one plant genus was also observed (Masicotte et al 1999). Results from field studies are somewhat contradictory. Although Horton and Bruns (1998) did not observe a high level of host specificity in the ECM colonizing the roots in a mixed forest stand of Douglas fir (*Pseudotsuga menziesii var. glauca*) and Bishop pine (*Pinus muricata),* a field study by Kernaghan et al. (2003a) showed that 30% of the most abundant ECM fungi exhibited host specificity and another 25% exhibited some level of host preference.

Therefore this study was undertaken to further our knowledge of the community structure of ECM fungi in boreal forest and to examine whether

distinct assemblages of fungi would be present in plots dominated by different ECM tree host. The objectives of the first chapter of the thesis were: i) to compare the ECMF species assemblages in 100m^2 plots dominated either by trembling aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marsh.) or white spruce/balsam fir (*Picea glauca* (Moench) Voss, and *Abies balsamea* (L) Mill.) from plots of 2 post-fire ages, ii) compare the results of the morphological and molecular characterization of the fungi. The hypotheses for this chapter were that the overall ECM diversity and richness between plots may be similar but that distinct fungal assemblages would be found in the plots of different canopy types. From the molecular characterization of the fungi, species richness was expected to be greater than that based on the morphological methods. Also, because of the finer resolution at the molecular level, associations between fungal types, canopy type, and age are more likely to be revealed using the data from the molecular analysis.

Arbuscular Mycorrhizae

Another widespread group of mycorrhizal associations is the endomycorrhizal fungi. There are several types of endomycorrhizae, all of which are characterized by the penetration of fungal hyphae into the plant root cells. The arbuscular mycorrhrizal fungi (AMF) are the most ancient and most widespread type of endomycorrhizal association, colonizing the roots of up to two-thirds of the world's vascular plant species (Newsham et al 1995). AMF are

a monophyletic group which have been placed into their own fungal phylum, the Phylum Glomeromycota (Schüßler et al 2001b).

AM fungi may play an integral role in plant community structure. Van der Heijen et al (1998) have shown that increased AMF diversity was correlated with increased plant diversity. Plants differ in their responses to different types of AMF and this high variation in plant growth responses to different AMF species may play an important role in the coexistence of plant species and the overall structure of plant communities (Klironomos 2003, van der Heijden et al 2003). Host plant species in turn may significantly alter the composition and structure of AMF communities. Host plants can influence fungal composition by regulating C allocation to roots, by producing secondary metabolites, or by changing soil environmental conditions. Burrows & Pfleger (2002) reported that plots with higher plant diversities produced from 30-150% more spores than plots with one plant species. Differences in AMF spore diversity, evenness and richness in relation to plant communities have been reported in several studies (Johnson et al 1992, Bever et al 1996, Eom et al 2000).

As it has been shown that different growth responses are seen with differing host-fungus combinations (Klironomos 2003), the identification of AMF species in the field is important for both the understanding of the ecology of the AM fungi and their plant hosts. Identification of AMF within an ecosystem has usually been based on the morphology of the asexual spores collected from the soil. However, molecular studies have shown that populations of spores in soil do not reflect the fungi present in roots (Clapp et al 1995). Using AMF specific

primers to amplify the fungal SSU rRNA gene, a greater knowledge of the diversity and the relationships between AMF and their host plants in their natural communities is being acquired (Helgason et al 1999, 2002, Husband et al 2002a, b, Vandenkoornhuyse et al 2002).

Relatively few studies have examined AMF biodiversity in Canada, and most are based on the presence of AM spores in the soil (Dalpé 2003). Information of AM communities in the boreal forests are also scarce, with only one study that has examined AMF diversity in a boreal Scots pine forest in Estonia (Opik et al 2003). Therefore, the first objective of the second chapter of the thesis was to examine the AMF diversity in *Clintonia borealis* from the boreal mixed-wood forest in Abitibi, QC. Sampling was conducted in the same plots as those used in the ECM study (Chapter 1) and *Clintonia borealis* was selected because it is present in all plots of each canopy type. Plants were collected in $100m^2$ plots dominated by birch, trembling aspen or a mix of white spruce and balsam fir in sites of 2 post fire ages. The second objective addressed in this study examined whether there is a change in fungal populations in plants collected under the different canopy types. Collecting plants in the boreal forest in Québec is of particular interest as it is an ECM dominated habitat and the investigation of AMF diversity in such a habitat has rarely been studied. The distribution of AMF types were expected to differ in plants collected under the different canopy types, and as molecular methods were used to analyze the AM fungi, I hypothesized that a relatively high richness of AM types would be detected.

Soil microfungi

Studies have shown that in nature, unique combinations of microfungal populations form with particular vascular plant communities (States 1981). Apinis (1972) states that taxonomic groups of fungi are potentially good indicators of habitat, as fungal communities reflect the conditions of the soil environment and the contributions of the herbaceous and woody components of the accompanying plant community.

The relationships between plant community and fungal assemblages have been shown in various studies. In 3 successional forest communities in Oklahoma, many fungal species were common to all 3 areas, indicating that most fungi tolerate a wide range of conditions, but the principal (most abundant) species in each area had distinct species compositions in the different forest types (Malik and Rice 1966). Mclean and Huhta (2002) attribute differences between fungal communities observed in anthropogenic birch stands, spruce forests, arable fields, and an old deciduous forest to the litter quality. Different leaf and wood litters differ in their chemical components and different plants release different root exudates that can affect the surrounding microbial community.

Distinct microfungal communities are usually observed between deciduous and coniferous forests. Coniferous forests differ from deciduous forests in a variety of ways. For example, coniferous forests have slower decay rates and more acidic soils, which may be just a few factors that may attribute to the differences in fungal communities found in these 2 forest types (Thornton

1956). Different types of coniferous forests also harbour different fungal communities. A study of the soil microfungal communities in Finland reports differences in microfungal communities between pine and spruce forests (Soderstrom 1975).

Investigations on the influence of plant community type on soil mycoflora from forest soils in southern Québec showed differences in abundances and habitat preferences of certain fungal genera between coniferous and deciduous forests (Widden 1986). The distributions of soil fungi are associated with particular soil environments containing suitable substrates that allow different species to thrive. Soil properties are largely influenced by the nature of the vegetation cover, thereby explaining the link that is usually seen between soil fungi and plant cover.

The symbiotic mycorrhizal fungi obtain their sources of carbon from their plant hosts thus their distributions are predicted to be more tightly associated with their plant hosts. Being dependant on organic substrates, the distributions of the microfungi within the plots are likely to be more dependant on the different microenvironments within the different plots. In fact, a study by Villenueve et al (1989) surveyed ECM and saprotrophic sporocarps along an environmental gradient of increasing environmental rigor and instability, and reported that the species richness of ECM fungi remained relatively constant while the species richness of the saprotrophic fungi declined. The mycorrhizal fungi receiving most of their energy from their plant hosts may be better buffered against the surrounding environmental fluctuations (Gehring et al 1998).

Thus the objectives of the third chapter were: i) to examine the microfungal community within plots supporting the range of boreal tree and understory plant species; and ii) examine the correlations between the microfungal assemblages with the aboveground tree cover and understory plants. In the data collected for this chapter, I expect distinct fungal assemblages in the plots of different canopy types. I also predict that the microfungi would have tighter associations with their immediate surroundings. Thus, although correlations between the microfungal community and overstory tree community were expected, stronger correlations between microfungal assemblages and the surrounding understory herbaceous layers were expected.

Chapter 1. Relationships between stand composition and ectomycorrhizal community structure in boreal mixed-wood forests

Abstract

We investigated the community structure of ectomycorrhizal fungi under varying overstory tree compositions in the southern mixed-wood boreal forest of Quebec. Sampling took place at two locations of differing post fire ages and nine $100m^2$ plots were sampled per location. The dominant overstory tree species in the plots were trembling aspen (*Populus tremuloides* Michx.), white birch (*Betula papyrifera* Marsh.), or white spruce (*Picea glauca* (Moench) Voss) and balsam fir (*Abies balsamea* (L.) Mill.). Mycorrhizae were analyzed using morphological as well as molecular methods, employing fungal-specific primers to amplify ribosomal DNA for subsequent cloning and sequencing. One thousand eight hundred mycorrhizal root tips collected from the 18 plots were morphologically classified into 26 morphotypes, with *Cenococcum geophilum* dominating (36% of root tips). A second set of root tips, selected from the same 18 samples on which the morphological analysis was based, were analyzed using molecular methods. From this analysis, 576 cloned PCR products were screened by restriction fragment length polymorphism analysis and a total of 207 unique types were found. No one type dominated the system and 159 occurred only once. Sequence analysis of the types that occurred more than once revealed that *Piloderma sp., Russula sp., Cortinarius sp.* and *Lactarius* sp. were the most common mycorrhizae. The ectomycorrhizal fungal community structure revealed

by the rDNA analysis differed from that observed using morphological methods. Canonical correspondence analyses of the sequenced restriction types and % overstory composition indicate that the distributions of ectomycorrhizal fungi differ with the relative proportions of host tree species. The distinct fungal assemblages found in the different plots supported by the different combinations of host tree species provides further support for the need to conserve stand diversity in the southern boreal forest.

Introduction

Mycorrhizal fungi play an integral role in forest ecosystems and are crucial for tree growth and forest productivity. Mycorrhizal fungi aid their hosts by increasing nutrient absorption, providing pathogen resistance and increasing tolerance to harsh conditions such as heavy metal toxicity and drought (Smith and Read 1997). Ectomycorrhizal (ECM) fungi colonize the fine roots of a vast majority of the dominant tree species in the world's temperate and boreal forests (Read 1991). A high diversity of ECM fungi may be necessary to maintain the stability and resilience of the forest ecosystem (Perry et al 1989). Both ECM fungal species diversity and composition have been shown to have important consequences for growth and nutrient uptake of the host plant (Baxter and Dighton 2001, Jonsson et al 2001). Thus, a highly diverse ECM community should more efficiently capture limiting resources and improve plant growth (Kernaghan 2005).

The factors influencing ECM fungal community structure and diversity are

poorly understood. This may partly be explained by the fact that most studies of ECM fungi focus on changes in ECM fungal community structure in relation to recent changes in their environment such as wildfire, increased pollution or forestry management practices. Few studies however, have examined the role of host species composition on ECM fungal diversity in relatively unmanaged forests. However, abiotic factors such as soil chemistry and microclimate (Erland and Taylor 2002, Gehring et al 1998), as well as biotic factors such as the proteolytic capabilities of the ECM fungi (Dahlberg et al 1997) and stand age (Visser 1995) have been shown to play important roles in determining the composition of ECM fungal communities. Differences in root growth characteristics of the host plant may also affect ECM fungal composition. For example, ECM fungal communities have been shown to differ among soil layers (Dickie et al 2002, Tedersoo et al 2003), and it is thought that different ECM fungi can colonize various locations along the root system, due to differences in available carbohydrates (Bruns 1995).

The species composition of ECM host plant communities also directly affects ECM community structure through host specificity (i.e. potential host range) or host preference (i.e. degree to which potential hosts are colonized) (Massicotte et al 1994, Massicotte et al 1999). Based on sporocarp production, Molina et al (1992) list 11 ECM fungal species specific to *Betula*, 13 to *Abies* or *Picea,* and 2 to *Populus*. Nantel and Newman (1992) also found a high correlation between ECM fungi (fruit-bodies) and host species in a mixed forest regardless of other soil characteristics. A study by Kernaghan et al (2003a)

showed that majority of the dominant ECM fungal morphotypes in boreal mixed-woods exhibited some level of host preference or specificity. Host preference/specificity of ECM fungi is likely due to the release of specific organic compounds by the host plant, either belowground in the form of root exudates, or aboveground in the form of leaf input (Conn and Dighton 2004, Kernaghan 2005).

The relationships between host species composition and ECM community structure have, in general, received little attention. Associations between ECM fungal fruit bodies and various plant compositions have been observed in other studies (Bills et al 1986, Villeneuve et al 1989), but species of ECM fungi actually colonizing the roots were not examined. Many ECM fungal species fruit cryptically, and when species do produce aboveground sporocarps, production of these structures is ephemeral and dependent on environmental conditions. Sporocarp production is therefore a poor indicator of fungi colonizing tree roots (Gardes and Bruns 1996).

The mixed boreal forest of eastern Canada is an ecosystem in which the ecological processes are controlled to a large extent by disturbances such as fire and pest outbreaks. These disturbances create a mosaic of different ages across the landscape resulting in high spatial heterogeneity in canopy composition (Bergeron 2000), providing a model environment for the study of ECM fungal community structure. ECM diversity has been studied in many coniferous forests in Canada and Scandinavia (Dahlberg 2001, Durall et al 1999, Hagerman et al

1999), but boreal mixed-wood forests have only recently been explored in this respect.

Kernaghan et al (2003a) studied patterns of ECM fungal diversity in the same boreal mixed-wood forests as the present study. They found that overstory diversity and ECM diversity were positively correlated and that mixtures of conifer and broadleaf tree species tended to support greater ECM fungal diversity than mixtures of broadleaf species. In that study, however, measures of ECM fungal diversity were based on morphology only. The present study employs morphological as well as DNA based methods to describe more precisely the ECM fungal species composition in this boreal mixed-wood ecosystem by comparing ECM fungal communities among different stand types and ages situated on similar soils. We hypothesized that due to the host preferences of ECM fungi, characteristic assemblages of ECM fungi would be found in association with the different host tree combinations.

Materials & Methods

Site description. The study area is located in the Lac Duparquet Research and Teaching Forest, in northwestern Québec (48°30′N, 79°20′W). This area is part of the western balsam fir – paper birch (*Abies balsamea* (L.) Mill – *Betula papyrifera* Marsh.) bioclimatic domain (Grondin 1996), which extends over the Clay Belt region of Québec and Ontario. The closest weather station to the study area is located at La Sarre, 35 km north of Lac Duparquet. The average annual temperature is 0.8ºC, daily mean temperature is –17.9ºC for January and 16.8ºC for July, and the average annual precipitation totals 856.8 mm (Environment

Canada 1993). By dendrochronological analysis, Bergeron (1991) and Dansereau and Bergeron (1993) determined that the stands used in the present study originated from fires that took place 82 to 135 years ago. In the early stages of succession, paper birch (*Betula papyrifera* Marsh.), trembling aspen (*Populus tremuloïdes* Michx.), or jack pine (*Pinus banksiana* Lamb.), dominate the forest. If stands are not subjected to any major disturbances, they become dominated by balsam fir (*Abies balsamea* (L.) Mill.) and white cedar (*Thuja occidentalis L.*) (Legaré et al 2001).

Sampling design. A total of 18 sample plots, each measuring 10 × 10 m, situated on similar clay deposits were selected from an existing design (Legaré et al 2001). Half of the plots were located in a forest that originated from a fire that occurred in 1870, and the other half were in a forest with a 1916-1923 fire origin. Analysis of the soil chemistry data measured from the FH horizon in each plot from a previous study by Légaré et al (2001), revealed no significant differences in pH, mineral N, exchangeable calcium, magnesium, potassium, and available phosphorus among plots dominated by aspen, birch or coniferous species, or between plots of different ages. Within both the 1870 and 1916 fire stands, 3 plots were selected in each of three forest canopy types; i) trembling aspen dominated, ii) white birch dominated, and iii) white spruce - balsam fir dominated. A plot was assigned to one of the three categories when the corresponding species or group of species exceeded 75% of the total basal area of that plot. Two birch plots in the 1916 fire stand were 30m apart, but all other plots of

similar canopy type were a minimum of 50 m apart Some plots of different canopy type within a stand were as close as 10 m apart. In all plots, dominant trees originated after fire, except for the 1870 aspen plots, which are a second cohort of aspen (Bergeron 2000). In August 2002, we re-analyzed the overstory composition of these plots to ensure that the data still reflected the overstory composition recorded in 1994. The upper canopy in each plot was still dominated (>75%) by either trembling aspen, white birch and spruce-fir, as recorded by Légare et al (2001), but a lower canopy layer of ~1.5 to 2 meters high was present in most plots. The % cover of each tree species in this secondary canopy was recorded for each plot by visual observation.

Morphological analysis of ECM fungi. In September 2003, three cores of organic soil (~10cm in depth) were taken with a 7.5 cm diameter corer from each of the plots. The three cores from each plot were pooled and stored at 4ºC. The roots from a sub-sample of this soil mixture were washed away from the soil. The washed roots were cut into 2 cm pieces and laid on a gridded square petri plate (INTEGRID, Becton Dickson Labware, Lincoln Park, NJ). Each square on the petri plate was numbered and a selection of random numbers was used to select 100 root tips from each plate. On average, ten root tips were selected from each square. Root tips that appeared senescent were removed prior to selecting the 100 root tips for the analysis. Once the 100 root tips were selected, they were analyzed using a dissecting microscope and separated by their macroscopic features such as color, texture, and shape. Each root tip was then

mounted onto a microscope slide in 5% KOH and observed at 200-400X magnification for further separation on the basis of microscopic features such as mantle pattern, hyphal morphology, presence or absence of clamp connections and cystidia (Agerer 1991, 1995, Goodman et al 1996, Ingleby et al 1990). Each distinct ECMF type was assigned a morphotype.

Molecular analysis of ECM fungi. From the cores described above, a second set of 100 randomly selected root tips from each plot were placed in separate 1.5 ml eppendorf tubes with liquid N and lyophilized in a Savant SC110A Speed Vac ® Plus. Each set of 100 dried root tips was then ground to a powder in liquid nitrogen and the DNA extracted using the QIAGEN Dneasy® Plant Mini Kit (QIAGEN Inc., Mississauga, ON). The ITS region of the ribosomal DNA as well as a portion of the 28S gene was amplified using the fungal specific primers ITS-1F (Gardes and Bruns 1993) and NL6C (Kernaghan et al 2003b). Three DNA dilutions were tested (undiluted, 1:10 and 1:100) to increase the likelihood of obtaining a strong amplification product. The reactions were carried out in a final volume of 50 µl and included 0.2 mM dNTPs, 25 pmols of each primer, and 2.5 units of Taq DNA polymerase. The thermal parameters used were similar to those cited in Gardes and Bruns (1993). The resulting PCR products were cloned using the p-gemT easy cloning kit (Promega Inc., Madison WI) following the manufacturer's instructions. Thirty-two positive clones of each sample were selected and re-amplified with the primer pair used in the initial PCR. Glycerol stocks for each positive clone were prepared and tubes were stored at - 80ºC. A

total of 576 cloned products were then digested with Hinf I, Nde II, and Taq I according to the manufacturer's instructions (Promega Inc., Madison WI). Restriction digests were loaded onto a 2% agarose gel containing 0.02% ethidium bromide. All 32 digests for each restriction enzyme were loaded onto one gel, thus 3 gels were run for each plot. Agarose gels were examined on a GeneGenius Bio-imaging system (Synoptics, Cambridge, England) with GeneSnap 4.00.00 software (Synoptics Ltd.), lengths of restriction products were measured using GeneTools 3.00.22 software (Synoptics Ltd.) and compared to a 100bp plus molecular marker (MBI-Fermentas Corp.).

Restriction fragment length polymorphism (RFLP) sorting, sequencing and sequence analysis. The 32 RFLP patterns obtained from each plot were initially separated on a per plot basis using GERM software (Dickie et al 2003) with the default settings. RFLP types that were considered similar by GERM were always checked visually by comparing band patterns on the actual gel photos. Once the analysis of each plot was complete, a data set containing only unique RFLP types was re-analysed using GERM. Once again, all RFLP types grouped by GERM were verified by visual examination of the band lengths and patterns on the gel photos. A representative bacterial culture from each RFLP type that occurred more than once was selected and streaked onto LB-amp (100ul/ml) agar plates and grown overnight at 37ºC. Examples of each RFLP type were re-amplified using ITS-1F and NL6C, and PCR products were sequenced using an ABI PRISM® 3730XL DNA Analyzer system at the McGill

University and Genome Québec Innovation Centre. Forward and reverse primers used in the initial PCR reaction were also used in the sequencing reaction. Forward and reverse sequences were assembled using PHRAP Multiple Sequence Assembly Analysis in Curatools™ (CuraGen Corporation, http://curatools.curagen.com/login_portal/index.htm).

To identify the fungal species yielding the most common RFLP types, sequences obtained from the clones were aligned using nucleotide-nucleotide BLAST (blastn) in Genbank in order to locate similar ECM fungal sequences to act as references. Very few matches for the ~400bp portion of the 28S gene were found, thus this region was removed from the analysis leaving the ITS1-5.8S-ITS2 region of the rDNA. The neighbour-joining algorithm using PAUP*4.0b10 (Swafford 2002), was used to infer the placement of our sequences relative to the reference sequences obtained from Genbank. ClustalX 1.81 (Thompson et al 1997) was used to align the 47 ECM fungal sequences from this study along with 63 of the highest scoring BLAST hit sequences from GenBank. The alignments were then manually adjusted using Bioedit Ver. 5.0.6 (Hall 2001). The tree was rooted with a *Glomus mosseae* sequence obtained from Genbank (Accession AY236334).

ECM RFLP Richness and Diversity. Ectomycorrhizal richness and diversity indices were calculated for each canopy type based on the number of morphotypes and on the number of different RFLP types found in each plot. Although RFLP types (Egger 1995) and morphotypes (Sakakibara et al 2002)

may not always equate to individual species, they were treated as operational taxonomic units in order to calculate Shannon diversity (Shannon and Weaver 1949) and richness indices.

Ordination analysis: Canonical correspondence analysis (CCA) (ter Braak 1986) was used to assess the relationships between ECM composition and overstory composition and it was also used to assess the relationships between the ECM compositions of plots of similar ages. Proportions of host tree species occurring on each plot were used as a secondary (environmental) matrix, constraining the CCA axes to be linear combinations of these variables.

Patterns in host preference were explored using correspondence analysis (CA) (Jongman et al 1995). This analysis was performed using a plot type-ECM RFLP type matrix that included data on the abundance of ECM RFLP types that occurred in more than one plot; therefore only 23 of the 47 ECM fungal RFLP types were used in this analysis. In the resulting CA diagram, the RFLP types closest to each canopy type represent the fungi most abundant in those plot types. Ordinations were performed using PC-ORD software (McCune and Mefford 1999).

Results

Morphological characterization of ECM fungi. Twenty-six different morphotypes were observed. The most common morphotypes were *Cenoccocum geophilum* (36% and found in all 18 plots), followed by an

unidentified Ascomycete (16% and found in 14 plots). The remaining ECM morphotypes include: Russulacaeous (12%), Thelephoraceous (9%), Cortinariaceous (7.5%), *Piloderma*-like (6%), *Amphinema*-like (5%), unknown Basidiomycetes (2.7%) and other unknown fungi (5.8%). The Russulacaeous morphotypes were present in 16 plots, the Thelephoraceous types were present in 13 plots, the Cortinariaceous were found in 10 plots, the *Piloderma*-like in 9 plots, and the *Amphinema*-like were found in 10 of the 18 plots. From this analysis, we found 3 morphotypes unique to the conifer dominated plots, 2 to the aspen dominated plots and only one morphotype was unique to the plots dominated by birch. The diversity indices were 2.1, 2.2, and 2.0, and ECM morphotype richness was 18, 18 and 20, for the birch, conifer and aspen dominated plots, respectively.

RFLP analysis of ECM fungi: richness and diversity. From the 576 clones screened, a total of 207 RFLP patterns were differentiated according to their Hinf I, Nde II and Taq1 patterns. The most common RFLP type occurred 65 times (11.3%), and was present in 5 out of the 18 plots. Ten other RFLP types occurred more than 10 times, 23 RFLP types occurred between 3 to 9 times, 15 types occurred twice, and 159 RFLP types occurred just once (27.6%).

Based on RFLP analysis, the birch plots had the highest mean diversity value $H' = 4.2$, the conifer plots had an intermediate value of $H' = 3.8$, and the aspen plots had the lowest diversity value, $H' = 3.0$. Richness values based on

molecular analysis are 106, 72 and 53, for the plots dominated by birch, conifer, and aspen (respectively).

Identification of fungi by sequence analysis. The primer set used in the molecular analysis does not discriminate between mycorrhizal and non-mycorrhizal fungi, thus all fungi can be amplified using this system. By selecting only colonized root tips we tried to focus on the ECM fungi, but it is inevitable that other non-mycorrhizal species may be amplified. 48 RFLP types occurred more than once and a representative sample of each of these was sequenced. All sequences obtained were used in the analysis and we did not disregard non-mycorrhizal taxa.

Blast searches in Genbank revealed that 47 of the 48 sequences amplified with ITS-1F and NL6C primers had high sequence similarity to members of the Ascomycota or Basidiomycota. One sequence was obviously chimeric and was removed from the analysis. It was composed of an ITS1 region with high sequence similarity to one ECM fungal species, and an ITS2 region with high similarity to another. When congeneric species from all plots are grouped together, *Piloderma* spp., *Russula, Cortinarius* and *Lactarius* spp., respectively, were the most common taxa, representing 75% of the sequenced clones. The neighbour-joining tree constructed with the remaining 47 sequences and reference Genbank sequences is shown in Fig. 1.

Relationships between ECM composition and overstory composition (based on the molecular analysis of the ECM fungi). CCA based on the 47 sequenced RFLP types and % overstory composition in all eighteen plots revealed that distinct ECM fungal assemblages were present in plots dominated by the different overstory trees (Fig. 2). The eigenvalues for the first three axes were 0.674, 0.591, and 0.449, and the intraset correlations for axis 1, % birch= -0.512 and % spruce= 0.378: axis 2, % birch= -0.720 and % spruce= 0.551, and axis 3, % aspen= 0.952 and % spruce= -0.739. The intra-set correlations are used to infer which environmental factors are contributing most to each axis (Jongman et al 1995). Hence, we can infer that axis one, which accounts for most of the variation, represents a birch-spruce gradient. The first 3 axes displayed strong species-environment correlations (axis 1, r=0.966, axis 2, r=0.931, axis 3, r=0.883), but they account for only 19.4% of the total variance in the species data with respect to the overstory tree assemblages (4 environmental variables). The CCA was also performed using the entire RFLP dataset (207 RFLP types) and % overstory composition, with similar results (not shown).

Differences between locations: Separate CCA analyses, based on the molecular characterization of the ECMF, from plots of the same fire origin (i.e. location), also show that plots of similar canopy type tend to cluster together (Fig. 3a and b). For the 1870 dataset, the first 3 axes of the CCA accounts for 39.3% of the total variance in the species data with respect to the overstory tree assemblages (intraset correlations for the 1870 plots were: axis 1, % spruce = -

0.925 and % birch = 0.542: axis 2, % fir = -0.639 and % birch = 0.578; the species-environment correlations were: axis1, r= 0.994; axis 2, r=0.937). The eigenvalues for the first two axes were 0.787 and 0.610. For the 1916 dataset, the first 3 axes of the CCA account for 49.4% of the total variance in the species data with respect to the overstory tree assemblages (intraset correlations for the 1916 plots were: axis 1, % spruce = -0.485 and % birch = 0.280: axis 2, % aspen = -0.971 and % birch = 0.769; the species-environment correlations were: axis1, r= 0.990; axis 2, r=0.979). The eigenvalues for the first two axes were 0.892 and 0.809. The main difference between the two bi-plots is a better separation between the birch and conifer dominated plots in the bi-plot based on the 1870 data. The birch and conifer dominated plots from the 1916 fire are not as distinct from each other as they are in the 1870 plots. The 1916 conifer plots contain approximately 20% birch in their upper canopies, while the 1870 conifer plots have less than 4% birch in their upper canopy. Also the 1916 birch plots have on average 18% balsam fir in the upper canopy and 38% balsam fir in their secondary canopy. Further analyses of differences between the plots of different ages were not attempted, as plots of similar post fire age are located in fairly close proximity, and therefore confounded by location.

ECMF Host preference: RFLP types representing *Piloderma, Lactarius, Russula, Cortinarius, Tylospora, Amphinema* and *Cenococcum* are not evenly distributed among the birch, conifer and aspen plots (Fig.4). The correspondence analysis using only ECM RFLP types that occurred in more than

one plot also showed that different RFLP types had different patterns of abundance in the plots of differing canopy type (λ_1= 0.40, λ_2 = 0.31, Fig. 5). Table 1 shows the number of plots in which each of the ECM RFLP types were found.

Figure 1. Neighbour-joining tree demonstrating the placement of the sequenced RFLP types with reference sequences obtained from Genbank. Sequences from Genbank are identified with their accession numbers. Sequenced RFLP types are shown in bold. Bootstrap values (1000 replicates) are shown at the nodes. The scale bar at the bottom left is proportional to branch length.

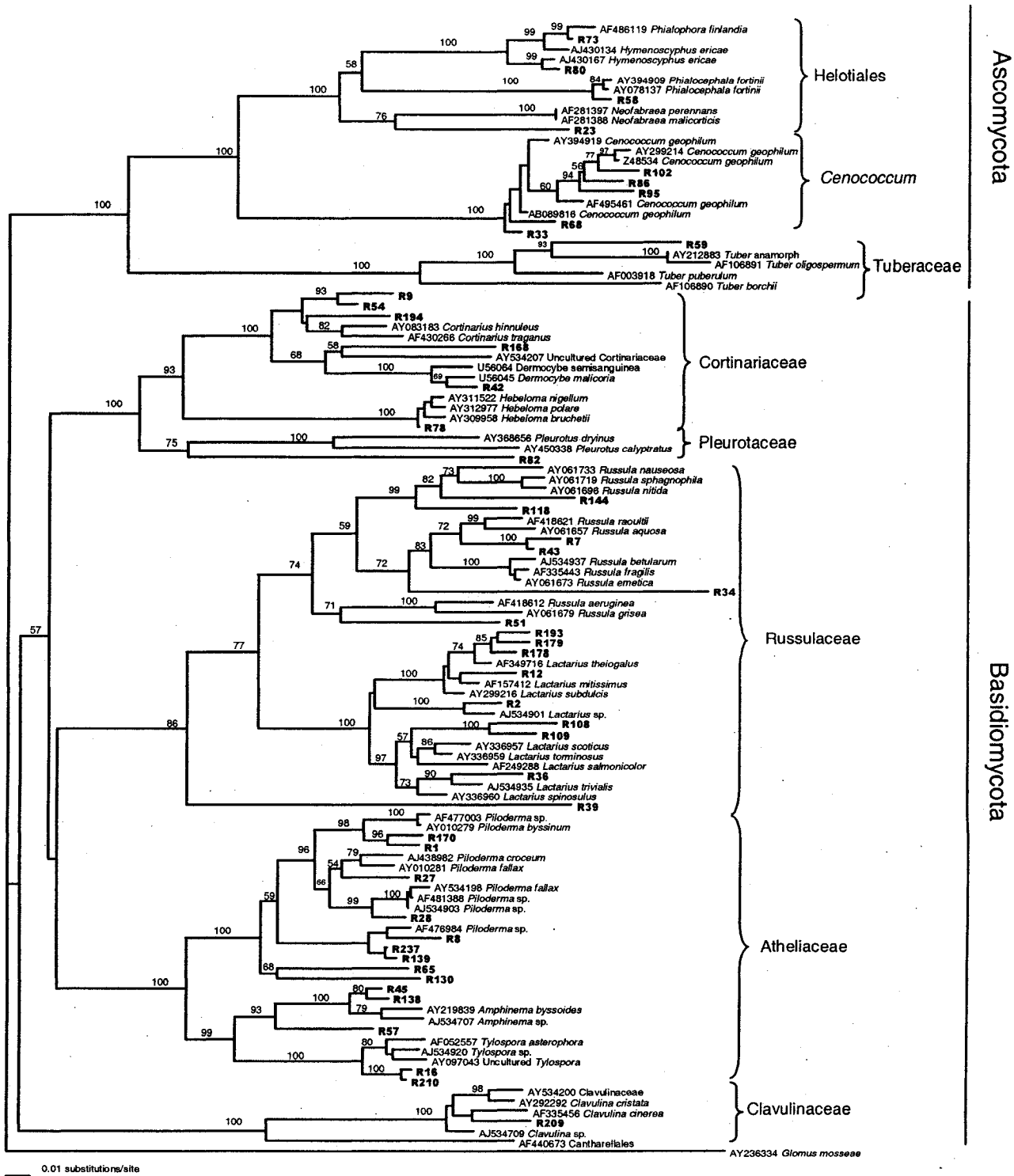
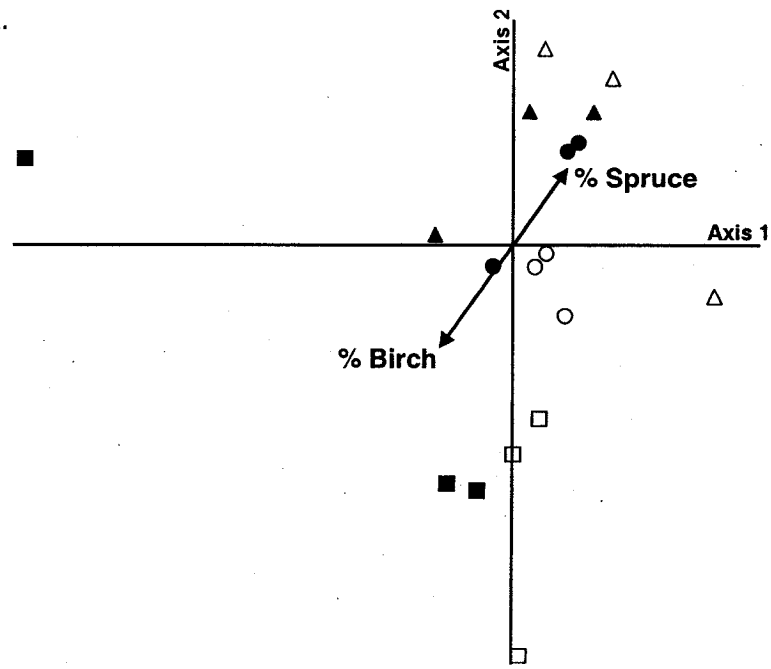

AF486119 Phialophora finlandia
R73
AJ430134 Hymenoscyphus ericae
AJ430167 Hymenoscyphus ericae
R80
AY394909 Phialocephala fortinii
AY078137 Phialocephala fortinii
R58
AF281397 Neofabraea perennans
AF281388 Neofabraea malicorticis
R23
Helotiales
AY394919 Cenococcum geophilum
AY299214 Cenococcum geophilum
Z48534 Cenococcum geophilum
R102
R86
R95
AF495461 Cenococcum geophilum
AB089816 Cenococcum geophilum
R68
R33
Cenococcum
R59
AY212883 Tuber anamorph
AF106891 Tuber oligospermum
AF003918 Tuber puberulum
AF106890 Tuber borchii
Tuberaceae
R9
R54
R194
AY083183 Cortinarius hinnuleus
AF430266 Cortinarius traganus
R168
AY534207 Uncultured Cortinariaceae
U56064 Dermocybe semisanguinea
U56045 Dermocybe malicoria
R42
AY311522 Hebeloma nigellum
AY312977 Hebeloma polare
AY309958 Hebeloma bruchetii
R78
Cortinariaceae
AY368656 Pleurotus dryinus
AY450338 Pleurotus calyptratus
R82
Pleurotaceae
AY061733 Russula nauseosa
AY061719 Russula sphagnophila
AY061696 Russula nitida
R144
R118
AF418621 Russula raoultii
AY061657 Russula aquosa
R7
R43
AJ534937 Russula betularum
AF335443 Russula fragilis
AY061673 Russula emetica
R34
AF418612 Russula aeruginea
AY061679 Russula grisea
R51
R193
R179
R178
AF349716 Lactarius theiogalus
R12
AF157412 Lactarius mitissimus
AY299216 Lactarius subdulcis
R2
AJ534901 Lactarius sp.
R108
R109
AY336957 Lactarius scoticus
AY336959 Lactarius torminosus
AF249288 Lactarius salmonicolor
R36
AJ534935 Lactarius trivialis
AY336960 Lactarius spinosulus
R39
Russulaceae
AF477003 Piloderma sp.
AY010279 Piloderma byssinum
R170
R1
AJ438982 Piloderma croceum
AY010281 Piloderma fallax
R27
AY534198 Piloderma fallax
AF481388 Piloderma sp.
AJ534903 Piloderma sp.
R28
AF476984 Piloderma sp.
R8
R237
R139
R65
R130
R45
R138
AY219839 Amphinema byssoides
AJ534707 Amphinema sp.
R57
AF052557 Tylospora asterophora
AJ534920 Tylospora sp.
AY097043 Uncultured Tylospora
R16
R210
Atheliaceae
AY534200 Clavulinaceae
AY292292 Clavulina cristata
AF335456 Clavulina cinerea
R209
AJ534709 Clavulina sp.
Clavulinaceae
AF440673 Cantharellales
AY236334 Glomus mosseae
0.01 substitutions/site
Ascomycota
Basidiomycota

Figure 2a and b. Canonical correspondence analysis of the ECM community (based on the molecular identification) from the 18 plots supporting the various stand types of 2 post fire ages. Circles represent the aspen plots, triangles represent the conifer plots and the squares represent the birch plots. Open symbols represent plots from the 1870 fire, while the black symbols represent plots from the 1916 fire. Fig. 2a. displays axes 1 and 2, Fig. 2b. displays axes 2 and 3.

a.


Axis 2
% Spruce
Axis 1
% Birch

b.

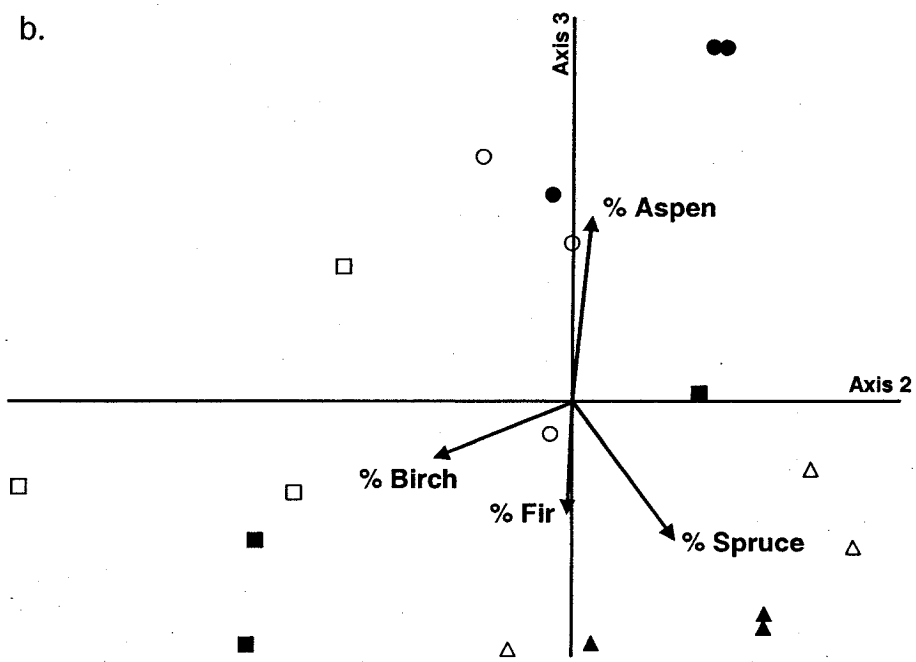
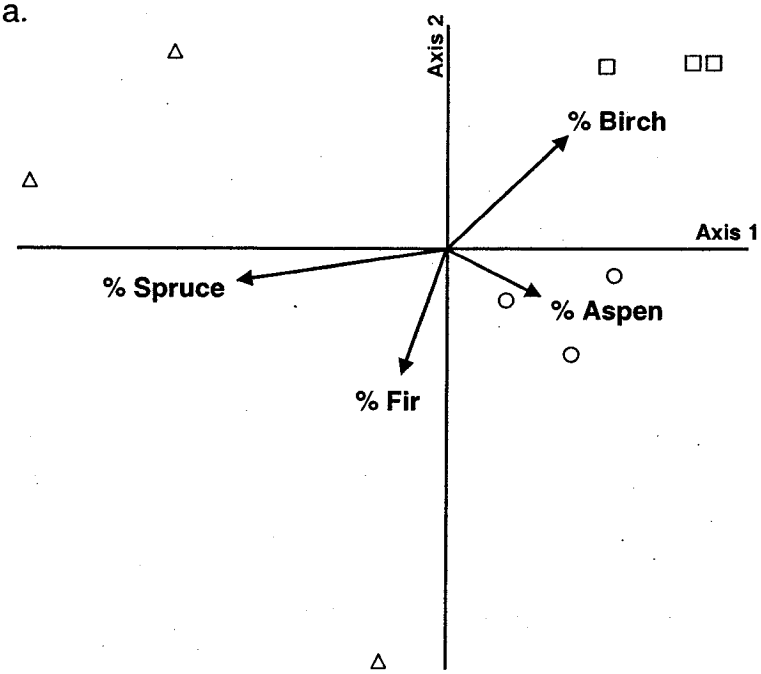
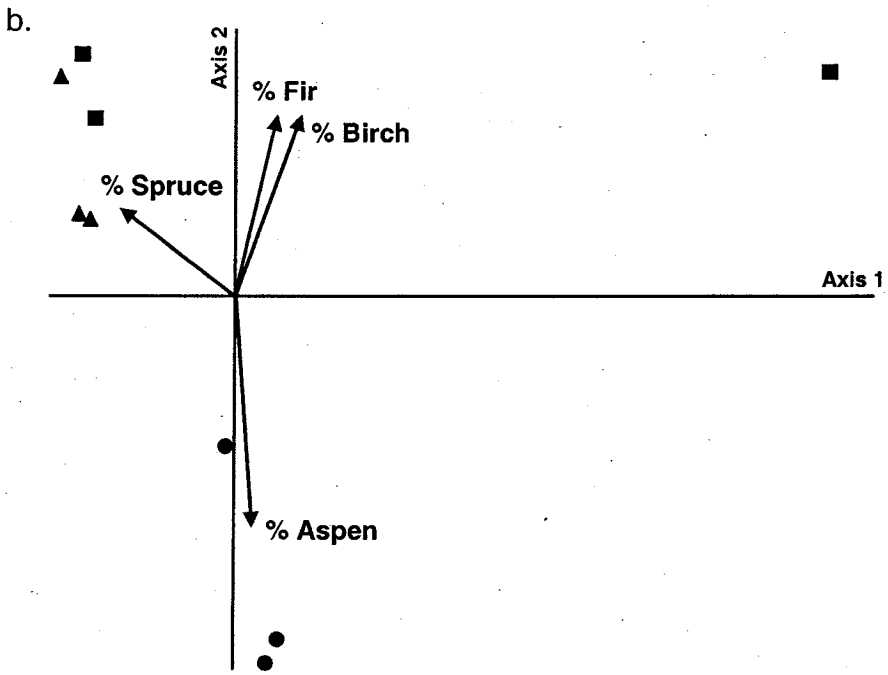

Axis 3
% Aspen
Axis 2
% Birch
% Fir
% Spruce

Figure 3a and b. Canonical correspondence analysis of the ECM community (based on the molecular classification) of plots of similar age groups supporting the various stand types. Circles represent the aspen plots, triangles represent the conifer plots and the squares represent the birch plots. Fig. 3a. displays axes 1 and 2 of the bi-plot of the 1870 plots, Fig. 3b. displays axes 1 and 2 of the bi-blot of the 1916 plots.

a.


Axis 2
% Birch
Axis 1
% Spruce
% Aspen
% Fir

b.


Axis 2
% Fir
% Birch
% Spruce
Axis 1
% Aspen

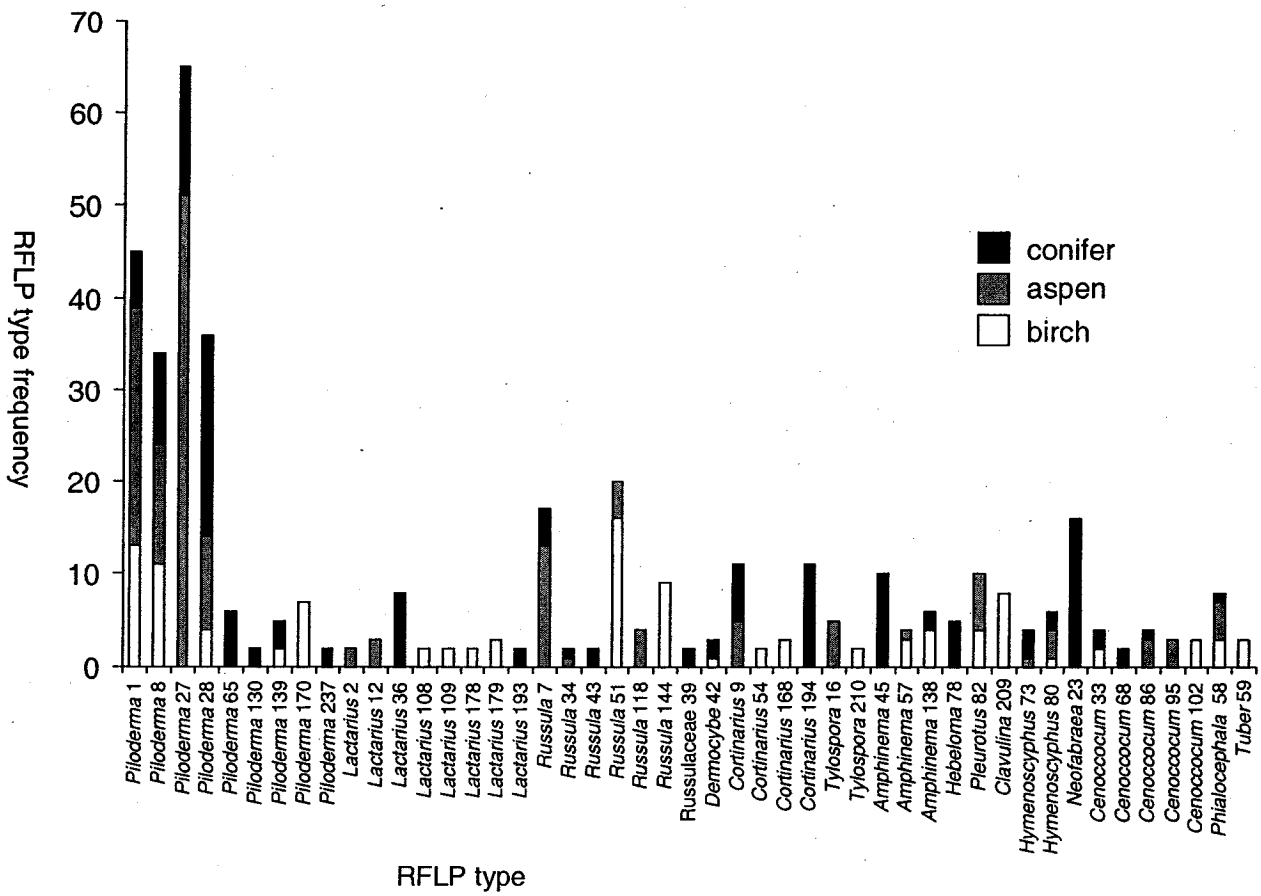


Figure 4. Frequency of each sequenced RFLP type per canopy type. Bars represent the number of times a certain RFLP type was found in the pooled dataset (from stands in both post fire ages) of plots of similar canopy type (n=6). Each sequence is assigned to a genus on the basis of its closest match in Genbank.

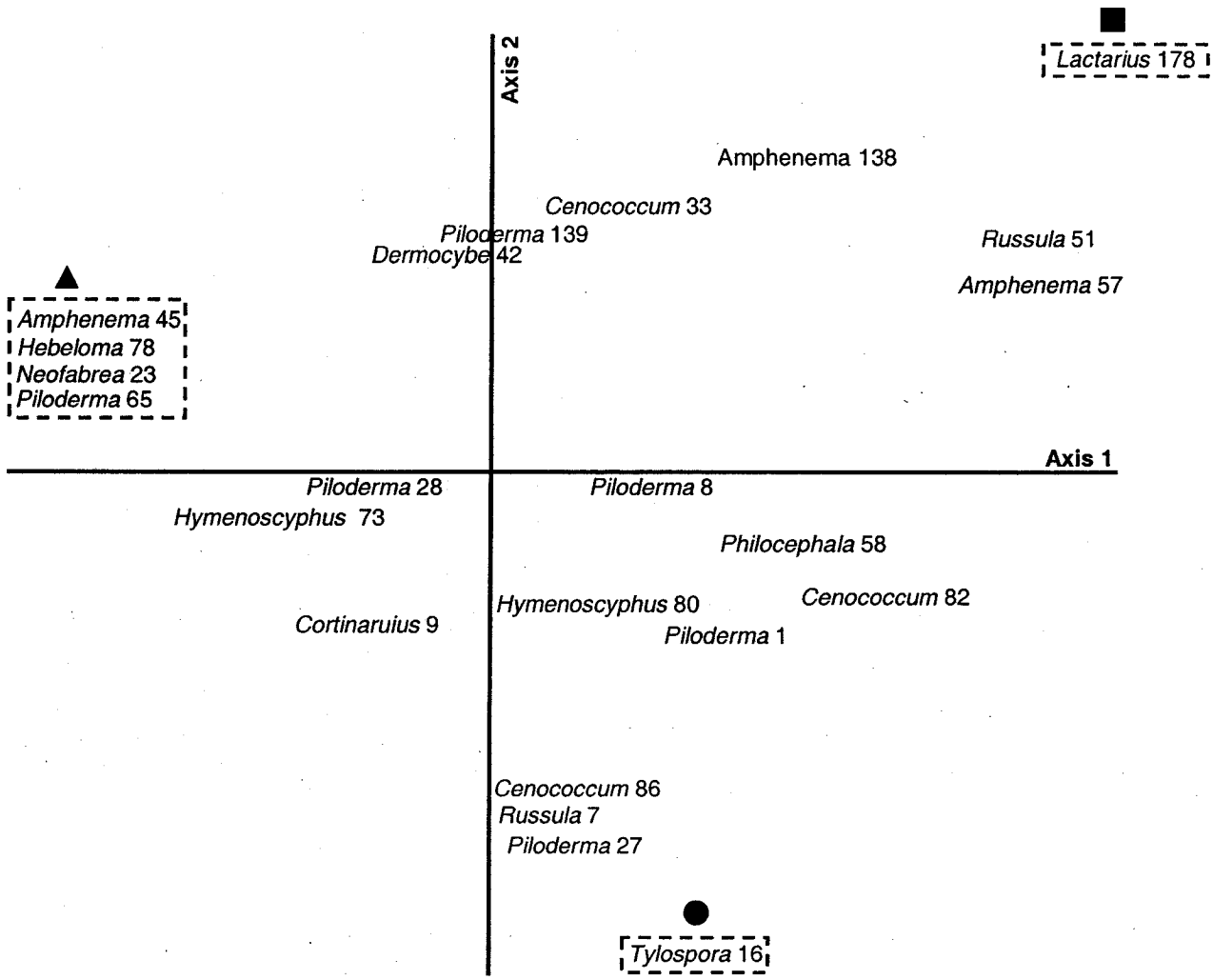


Figure 5. Correspondence analysis of the 3 canopy types and sequenced RFLP types that occurred in more than one plot. Boxed data represents types that were found exclusively in plots of a single canopy type. The circle represents aspen dominated plots, the triangle represents conifer dominated plots and the square represents birch dominated plots.

Table 1. Number and type of plots in which the most common ECM RFLP types were found

	Number of times found in plots dominated by:		
ECM RFLP type	birch	conifer	aspen
Amphenema 45	0	4	0
Amphenema 57	1	0	1
Amphenema 138	1	1	0
Cenococcum 33	1	2	0
Cenococcum 82	1	0	1
Cenococcum 86	0	1	2
Cortinarius 9	0	2	1
Dermocybe 42	1	1	0
Hebeloma 78	0	2	0
Hymenoscyphus 73	0	2	0
Hymenoscyphus 80	1	1	1
Lactarius 178	2	0	0
Neofabrea 23	0	2	0
Philocephala 58	2	2	1
Piloderma 1	1	0	1
Piloderma 8	3	3	2
Piloderma 27	0	3	2
Piloderma 28	0	2	2
Piloderma 65	0	2	0
Piloderma 139	1	1	0
Russula 7	0	1	3
Russula 51	1	0	1
Tylospora 16	0	0	2

Discussion

Our data highlight the influence of overstory composition on ECM fungal community structure, as evidenced by the relative similarity of ECM fungal communities on plots of similar canopy type (Fig. 2 and Fig. 3a and b). Although the canonical relationships between overstory tree composition and ECM fungal species composition explained only a small amount of the variation, all 3 CCA biplots showed similar trends with % birch and % spruce accounting for most of this variation. The influence of the different tree species on the ECM fungal community is further emphasized in the separate CCA analyses of plots of similar age. The birch and conifer plots of the 1870 fire (Fig. 3a) form distinct clusters while the 1916 birch and conifer plots tend to cluster together (Fig. 3b). The different fungi found in these plots do show preference among the plots of different canopy type (Figs. 4 and 5), thus, the cluster of the 1916 birch and conifer plots may be a result of the of the relatively high conifer component in the 1916 birch plots.

Differences in the ECM fungal community found under the different host tree species are likely due to patterns of ECM fungal host preference (Massicotte et al 1994), brought about by differences in root and litter inputs (Conn and Dighton 2004, Kernaghan 2005), or perhaps to differences in patterns of belowground resource allocation (see Bauhus and Messier 1999). Providing explicit evidence for host specificity is beyond the scope of the current study, as we did not identify the roots from which the ECM fungi were obtained. However, our data indicates that certain ECM fungi exhibit preferences for certain stand

types (Figs. 4, 5). Not only did the birch dominated plots appear quite distinct, with most plots clustering together in the bi-plots (Fig. 2), they also support the highest ECM fungal RFLP richness and diversity. Other studies have also reported distinct microbial communities in birch soils; Smolander (1990) has shown increased densities of *Frankia* sp. and DeLong et al (2002) report increases in pseudomonads in birch soils, and Priha et al (1999) found more fungal specific fatty acids in birch rhizosphere soils versus those of pine and spruce. Suitable carbon sources are thought to be the most limiting factor to soil microbes (Wardle 1992a), hence the increased ECM fungal richness and diversity may be related to the easily leached water soluble compounds released from birch litter (Berg and Wessén 1984) and to the root exudates of birch roots which have been reported to have high amounts of carbohydrates (Smith 1976). Studies of mixed conifer-birch stands report improved soil conditions (Brandtberg et al 2000) and enhanced productivity of the co-occurring conifers (Mard 1996), which may in part be due to the influence of birch on the soil microbial community. Based on studies of fruit-body production, birch is known to support characteristic ECM fungal species (Trappe 1962), but, to our knowledge, our study is the first to demonstrate that the ECM fungi actually colonizing the roots of birch form a distinct and relatively diverse community.

Spruce also exhibited an influence on the ECM fungal community. Trappe (1962) lists several *Picea* specific ECM fungal species (on the basis of fruiting bodies) and Molina and Trappe (1982) demonstrated differences among ECM fungal species in their ability to colonize different conifer species *in vitro.* Koide et

al (1998) and Baar and de Vries (1995) suggest that the chemistry of forest litter can affect ECM fungal communities. Conifer litter contains high levels of phenolics (Strack et al 1988), and decomposes relatively slowly (Pastor et al 1987), resulting in an accumulation of recalcitrant forest floor material (Paré and Bergeron 1996), which may influence the ECM fungal community. Although fir is also known to support characteristic ECM fungal species (Kernaghan et al 1997, Molina and Trappe 1982, Trappe 1962), only a small amount of the variation in ECM species composition was explained by the presence of fir. However, the mean percent basal area occupied by fir in the conifer plots was only 15%.

Aspen exhibited the least influence on the structure of the ECM fungal community and its influence varied between the two locations studied (Figs. 3a, b). Although aspen is associated with some unique ECM fungal species (Cripps and Miller 1993, Molina et al 1992), their roots have been shown to support a lower ECM fungal diversity than any other host tree in our study area (Kernaghan et al 2003a). The aspen trees in our study area represent the second post-fire cohort and are therefore younger than the other host trees. However, aspen can survive belowground after fire and quickly re-colonize by forming root suckers, potentially making the aspen root system older than that of the other host trees.

CCA analysis based on the morphological characterization of the ECM fungal community and overstory composition (not shown) reveals a similar, yet less obvious pattern of ECM fungal distribution, with plots of similar canopy type clustering less tightly than in the RFLP based CCA. In general, the molecular methods afforded a much finer level of resolution by detecting intraspecific

variation in the ITS region of the ECM fungi (Fig. 1). This allowed for the detection of greater levels of host preference, and therefore a more accurate picture of the differences among ECM communities from different stand types (Fig. 4).

The CA diagram (Fig. 5) shows the distributions of the ECM fungal species in relation to the host tree species and indicates that some RFLP types exhibit preference for certain plot types. For example, *Russula* (RFLP #51) and *Amphinema* (RFLP #78), were most abundant in birch plots. *Piloderma* (RFLP #65), *Hebeloma* (RFLP #78), *Amphinema* (RFLP #45) and *Neofabrea* (RFLP #23) were found only in conifer plots, *Tylospora* (RFLP #16) was present only in the aspen plots and *Lactarius* (RFLP #178) was found only in the birch plots. ECM types that clustered in the middle of the diagram were those that were found in all plot types, such as *Pilodema* (RFLP #8) and *Hymenoscyphus* (RFLP #80). The first axis of the CA diagram explains the greatest amount of variation and, as in the CCA diagrams, shows a conifer deciduous separation.

Another difference between the results obtained using the morphological and molecular characterization of the ECM fungi is that the dominant ECM types revealed by the morphological analysis were darkly pigmented types such as *Cenococcum geophilum* and the thelephoroid fungi. The morphological analysis in the present study, and that of Kernaghan et al (2003a) (an analysis of the ECM fungi in the same plots from the 1916 fire using morphological methods) revealed that *Cenococcum* and species within the Thelephoraceae dominated. However, our molecular data show that very few clones had sequence similarities to

Cenococcum geophilum and none of the sequenced clones matched thelephoroid fungi. Both *Cenococcum geophilum* and members of the Thelephoraceae form robust mantles and are easily identified morphologically (Goodman et al 1996, Koljlag et al 2000). This may allow for their identification even after the fungal or host tissues are dead (Valentine et al 2004). Thus, morphological analyses may overestimate the abundance of ECM with distinctive and persistent mantles. The DNA of fungi that possess dark, melanized hyphae may also not be as effectively extracted as that of other fungi. The lack of sensitivity with respect to these fungi could also be due to primer bias, but the same primer pair was also used by Kernaghan et al (2003b) who successfully amplified *Cenococcum* and thelephoraceous fungi from mycorrhizal plants growing in nurseries. The observed differences between the morphological and molecular methods implies that the best approach for accurate assessment of ECM fungal communities may be to combine the two methods in a stratified approach, such as that employed by Sakakibra et al (2002), who used a primary morphological assessment, followed by molecular analysis to differentiate ECM morphotypes to species.

The molecular analysis revealed 207 RFLP types, with only a few RFLP types occurring with relative abundances greater than 5%. Dominance of a few species is a common feature of ECM fungal communities (Erland and Taylor 2002, Horton and Bruns 2001). By sequencing all RFLP types that occurred more than once, we were able to identify 72% of the clones, with Piloderma, *Russula, Cortinarius* and *Lactarius* species dominating. Apart from our low

recovery of *Cenococcum* and thelephoraceous sequences, this is in general agreement with other studies of ECM fungal communities from boreal forests (Dahlberg 2001).

Piloderma spp. are often common in old growth coniferous forests (Dahlberg et al 1997, Goodman and Trofymow 1998) and their occurrence has been correlated with coarse woody debris abundance (Smith et al 2000). The likelihood of finding *Piloderma* spp. in old-growth boreal forests may also be related to their enzymatic capabilities. Nitrogen availability in boreal forests is generally a limiting factor, and *Piloderma* spp. produce proteolytic enzymes that allow them to mobilize and take up N from organic compounds (Dahlberg et al 1997). This ability may be an important factor explaining the dominance of this fungus in this ecosystem, as N availability is low in both the 1916 and 1870 stands (Paré and Bergeron 1996). *Russula* species are also late successional ECM fungi (Deacon and Fleming 1992) that are known to have the potential to release peroxidases and/or polyphenol oxidase (Agerer et al 2000).

Our results indicate that overstory tree species composition is related to the belowground community of ECM fungi, possibly due to host preferences of the different fungi. Also, the differences between the data collected using morphological and molecular methods indicate that the use of either method alone may not give an accurate representation of the ECM fungal community. Although studies including more replication would be desirable to confirm the trends found in this study, our results provide further argument for the conservation of tree species diversity in the mixed stands of the southern boreal

forest. Regeneration after a disturbance such as fire or a clearcut is typically monospecific with gradual development of mixed stands over time (Bergeron and Harvey 1997). However forestry practices in this area commonly avoid the deciduous phase by planting coniferous trees, or are managed to encourage domination by fast growing trembling aspen (Bergeron and Harvey 1997). Forestry practices that strive to dramatically change overstory tree species compositions may therefore forfeit the potential for increased productivity in regenerating tree species brought about by colonization of host specific and functionally compatible mycorrhizal symbionts.

Acknowledgements

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Chapter 2: Diversity of the SSU rRNA gene of the arbuscular mycorrhizal fungi colonizing *Clintonia borealis* from a mixed-wood boreal forest.

Abstract

Molecular methods were used to investigate the community of arbuscular mycorrhizal fungi (AMF) in the roots of *Clintonia borealis* from a boreal mixed forest in northwestern Québec. *Clintonia* roots were sampled from 100m^2 plots dominated by three different overstory tree species, trembling aspen (*Populus tremuloides* Michx.), white birch (*Betula papyrifera* Marsh.), or a mix of white spruce (*Picea glauca* (Moench) Voss) and balsam fir (*Abies balsamea* (L.) Mill.). An AMF specific primer was used to amplify a portion of the 18S ribosomal gene and resulting PCR products were cloned. From 576 clones that were screened by restriction fragment length polymorphism analysis, a total of 92 different restriction patterns were found and sequenced. Fifty-two sequences closely matched other *Glomus* sequences from Genbank. Phylogenetic analysis revealed 10 different AMF sequence types, most of which clustered with other uncultured AM sequences obtained from plant roots collected from various field sites. Compared with other AMF communities investigated, using similar methods, richness and diversity in this boreal forest was higher than that observed in an arable field, but was lower than that seen in a tropical forest and a temperate wetland. No significant difference between the AMF communities from *Clintonia* roots under the different canopy types were observed, and the dominant sequence type represented 66.9% of all the clones analyzed. The

dominant sequence type clusters with AM sequences collected from a variety of environments and hosts at distant geographical locations.

Introduction

The arbuscular mycorrhizal fungi (AMF) form an ancient and widespread type of endomycorrhizal association found on the roots of up to two-thirds of the world's vascular plant species (Newsham et al 1995). This mycorrhizal symbiosis is one of the most important plant-microbe associations. The AMF aid their hosts by improving nutrient and water uptake, increasing pathogen resistance and enhancing stress tolerance (Smith and Read 1997). AMF also play an integral role in determining plant community structure (van der Heijden et al 1998). Plants differ in their responses to different AMF types and AMF differ in their ability to distribute nutrients between coexisting plants. This high variation in plant growth responses to different AMF species is thought to be a controlling factor in the overall structure of plant communities (Klironomos 2003, van der Heijden et al 2003). Despite their global distribution and ecological importance, knowledge of the community structure of AMF fungi is, however, scarce.

AMF are obligate symbionts and cannot be grown in culture without their host. Due to the difficulties of culturing the AMF from plant roots, the identification and quantification of AMF within an ecosystem has usually been based on studies of the asexual spores collected from the soil. Such studies have shown that host plant communities can influence the fungal community composition (Eom et al 2000, Johnson et al 1992). However, there are many

problems associated with using spores to analyze AMF communities. For example, recent advances in our understanding of the phylogeny of the Glomeromycota based on DNA sequences have demonstrated that some highly divergent taxa are not distinguishable by their morphological characteristics (Morton and Redecker 2001). Hence, studies of the AMF based on morphological characters of spores alone may not only leave many species unresolved, but, even when they can be identified, basing our understanding of AMF communities on spores in the soil is much like basing studies of plant communities only on the seed bank available. It is, therefore, not surprising that molecular studies have shown that populations of spores in soil do not reflect the fungi present in roots (Clapp et al 1995, Rosendahl and Stukenbrock 2004). Using the AM1 primer (Helgason et al 1998), which targets SSU rRNA gene of AMF, we are now able to detect the DNA of the fungi actually colonizing plant roots.

Using SSU rRNA gene sequences to understand AMF community structure also has its limitations, as it is known that different nuclei within a single isolate can contain different copies of the SSU rRNA gene (Hijri and Sanders 2005, Sanders 2002). However, Schüßler (1999), and Schüßler et al (2001a) reports that the within isolate variation of the 18S SSU gene is relatively small and AMF phylogeny has been based on this gene (Schüßler et al 2001b). Moreover, this method allows for the comparison of the genetic variation of the AMF found in this study with that from other ecosystems based on the same gene. To date, data on the genetic variation of the AMF using the AM1 primer is

probably the largest data set available on the genetic diversity of AMF collected from diverse natural environments.

Using SSU RNA, Helgason et al (1999) showed that the AMF of bluebell (*Hyacinthoides non-scripta* (L.) Chouard ex. Rothm) roots collected under oak were dominated by *Acaulospora* spp. whereas those collected from under sycamore canopies were dominated by *Glomus* spp. This was one of the first studies to show that the frequency of AMF sequences collected from a single plant host differed under differing canopy types. Molecular studies have also shown that different AM taxa are present in the roots of plants growing in different environments. Helgason et al (1998) and Daniell et al (2001) have shown that the AMF diversity in plant roots collected from an arable field was lower than that from a near-by woodland, and Helgason et al (1998), also showed that 92% of the sequences from crop plants were from *Glomus mosseae,* a species rarely found in the woodland system. Another common finding from molecular studies of AMF is that many of the sequences collected from the field do not match sequences from known, pot-cultured AMF. Thus, currently, sequences are partitioned into groups based on their similarities and cannot be assigned to definite species.

The mixed boreal forest of eastern Canada is an ecosystem in which the ecological processes are controlled to a large extent by disturbances such as fire and pest outbreaks, creating a landscape with a high spatial heterogeneity in canopy composition. In the Abitibi region of Québec, this results in a mosaic of patches dominated by either angiosperms such as birch and poplar, or by

conifers such as pine, spruce or fir (Bergeron 2000), all of which are ECM hosts. Legaré et al (2001) have shown that the understory plants, most of which are AM hosts, differ in community composition among these different canopy types, though some species, such as *Clintonia borealis* (Ait.) Raf. and *Aralia nudicaulis*, can be found throughout.

From the results of previous studies on AMF communities (Helgason et al 1999) we predicted that different AMF sequence types would be found in the roots of *Clintonia* plants collected from under the different canopy types. With the large genetic variation reported in the AMF, we also expected to find many novel sequence types in the *Clintonia* roots collected in this previously unexplored ecosystem.

We therefore undertook this study in order to obtain basic knowledge about the community composition of AMF in *Clintonia borealis* (Ait.) Raf. growing in a natural boreal mixed-wood forest. Our specific objectives were (i) to examine the AMF SSU rRNA sequence diversity from *Clintonia borealis* roots collected from a boreal mixed-wood forest and to compare the AMF sequence diversity and richness with those of other studies using the same primer pair, and (ii) to test whether the AMF community in the *Clintonia* roots would differ in plants collected under birch, aspen or conifer dominated canopies, and whether the AMF community would differ in sites of different post-fire ages.

Materials and Methods

Site description. The study area is located around Lake Duparquet, in northwestern Québec (48°30′N, 79°20′W). This area is part of the western balsam fir – paper birch (*Abies balsamea – Betula papyrifera*) bioclimatic domain (Grondin 1996), which extends over the Clay Belt region of Québec and Ontario. The closest weather station is located at La Sarre, 35 km north of Lake Duparquet. The average annual temperature is 0.8°C, daily mean temperature is –17.9°C for January and 16.8°C for July, and the average annual precipitation totals 856.8 mm (Environment Canada 1993). By dendrochronological analysis, Bergeron (1991) and Dansereau and Bergeron (1993) determined that the stands originated from fires that took place 34 to 281 years ago.

Sampling design. A total of 18 sample plots, each measuring 10 × 10 m, were selected on sites with similar clay deposits from an already existing design in the Lake Duparquet Research and Teaching Forest (Legaré et al 2001). Plots were located in stands of two different age classes, originating either from fires that took place in 1870, or in1916/1923. Within each stand age, 3 replicate plots of 3 different forest canopy types were selected. Plots were categorized as trembling aspen (*Populus tremuloïdes* Michx.), paper birch (*Betula papyrifera* Marsh.), or white spruce - balsam fir (*Picea glauca* (Moench) Voss - *Abies balsamea* (L.) Mill.) if the dominant species occupied at least 75% of the total basal area. In all stands, dominant trees originated after fire, except for the aspen stand of 1870,

which is a second cohort of aspen (Bergeron 2000). At each stand a total of 9 plots were sampled (3 x 3 canopy types).

Overstory, shrub layer and understory vegetation analysis. The overstory composition within the 100m^2 plots was originally recorded in 1994 (S. Legaré, pers. comm.). In August 2002, we re-analyzed the overstory composition of these plots to ensure that the data still reflected the overstory composition within the plots. At the time of sampling, a shrub layer of ~1.5 to 2.5 m was present in most of the plots. By visual observation, the % cover of each tree in this shrub layer was also recorded for each plot. In order to assess the understory herbaceous layer, a 1 x 1m subplot was set up in each of the eighteen 100m^2 plots, and the percent cover of each species in each subplot was estimated.

Sampling. In July 2002, three *Clintonia borealis* plants were selected from each of the 18 plots. Plants and attached root systems were carefully removed from the plots with a trowel. Soil was left around the roots to avoid desiccation and the whole plant was placed in a Ziploc© bag. The collected plants were stored in a cooler with ice packs, and were placed in a fridge at 4°C once they arrived at the laboratory. In the laboratory, roots of *C. borealis* were thoroughly washed with tap water followed by a 30-second wash in 30% H_2O_2 and a final rinse in distilled water. All root samples were visually inspected to ensure that they were living and healthy. A portion of each root system was placed in a separate clean 1.5 ml eppendorf tube and vacuum dried.

Molecular analysis. Equal portions of dried root samples of each of the 3 plants collected in each plot were combined and DNA was extracted from this root mixture. In a mortar, liquid nitrogen was poured over the root samples and the roots were crushed with a pestle. Three hundred μl of CTAB lysis buffer (Gardes and Bruns 1993) containing 0.02% b-mercaptoethanol were added to the mortar and this solution containing the crushed root sample was aspirated into a 1.5 ml eppendorf tube. One hundred μl of a 200ug/ml solution of proteinase K in TE buffer with 5% lauryl sulfate sodium salt was added to the sample and incubated for 1 h at 64°C. One volume of Tris-saturated phenol:chloroform:isoamyl alcohol (24:24:2) was then added and the tubes were mixed gently and centrifuged at 13000 x g for 15 min. The aqueous phase was placed into a clean eppendorf tube and the chloroform:isoamyl alcohol step was repeated. After centrifugation, the aqueous phase was placed into a clean tube and 100μl of a 50μg /ml solution of RNAse in TE buffer (Sambrook et al 1989) were added and the samples were incubated for 1 h at 37°C. The phenol:chloroform:isoamyl alcohol and chloroform:isoamyl alcohol separation steps were repeated. The aqueous phase was placed into a clean tube and 0.8 volume of cold isopropanol was added. To this solution 0.1 volume of sodium acetate (3M, pH 5.2) was added and the samples were kept at –20°C for 1 h. The samples were then centrifuged at 13000 X g for 15 min and the supernatant was discarded. Eight hundred μl of cold 70% ethanol were added to wash the pellet and the samples were centrifuged at max speed for 15 min. The supernatant was removed and samples were dried at room temperature. DNA

was re-suspended in 50μl of sterile ultra-pure water and incubated at 37ºC for 1h. After being re-suspended the samples were stored at 4°C. DNA was extracted from 18 different root mixtures. Partial SSU RNA fragments were amplified using a universal eukaryotic primer, NS31 (Simon et al 1992), and AM1, a primer that has been shown to amplify 3 families of the AM fungi (Glomeraceae, Gigasporaceae, and Acaulosporaceae (Helgason et al 1998)). A few DNA dilutions were tested (undiluted, 1:10 and 1:100) to increase the likelihood of obtaining a strong amplification product. Controls with no DNA were run with every series of amplifications to test for the presence of contaminants. The reactions were carried out in a final volume of 50 µl in the presence of 0.2 mM dNTPs, 25 pmols of each primer, and 2.5 units of Taq DNA polymerase. The thermal parameters used were similar to those cited in Gardes and Bruns (1993). The PCR parameters were initially tested using DNA extracted from *Glomus intraradices* spores obtained from Premier Tech Ltd. (Rivière du Loup, Qc.), and from DNA extracted from *Clintonia borealis* roots from a nearby forested area to ensure AMF DNA would be amplified. Based on these tests, the annealing temperature was raised to 58°C.

The resulting PCR products were cloned using the p-GEM-T easy cloning kit (Promega Inc., Madison WI). Thirty-two positive clones of the desired length (~550bp) were selected and re-amplified with the same primer pair as in the initial PCR for each DNA sample. A total of 576 cloned products were then digested with Hinf I, Nde II, and Taq I according to the manufacturer's instructions (Promega Inc., Madison WI).

Morphological analysis. If available, the remaining fine roots from each individual plant were stored in formalin-acetic acid for the morphological examination of the AM colonization. Roots were stained with 0.1% Chlorazol black E (De Bellis et al 2002) and stained roots were mounted on microscope slides in glycerine jelly (Widden 2001). The presence or absence of fungal material (AMF hyphae, vesicles or arbuscules) was recorded using a gridline intersect method (MCGonigle et al 1990) and the percentage of colonized intersections was calculated.

RFLP analysis and taxonomic analysis. The 32 RFLP patterns obtained from each plot were initially separated on a per plot basis using GERM (Dickie et al 2003). Once the analysis of each plot was complete, a data set containing all unique RFLP patterns from all 18 plots was re-analysed. From this analysis 92 unique RFLP types were found, one of which was extremely dominant. Examples of each RFLP type and 39 samples of the dominant RFLP type were re-amplified, and PCR products were sequenced using an ABI PRISM® 3730XL DNA Analyzer system at the McGill University and Genome Québec Innovation Centre.

To identify the RFLP types found, sequences were initially aligned using nucleotide-nucleotide BLAST (blastn) in Genbank in order to locate similar AMF sequences to act as references. The neighbour-joining (NJ) algorithm and maximum parsimony (MP) procedures, using PAUP*4.0b10 (Swafford 2002), were used to infer the placement of our sequences relative to the reference

sequences obtained from Genbank. Distances for the NJ tree were computed using the Kimura 2 parameter model. For the MP analysis a heuristic tree search with tree bisection and reconnection (TBR) as the swapping algorithm using 500 replicates with a random addition order of sequences was used to find the shortest tree. Bootstrap values for branches were estimated from 500 replicates for the MP analysis and from1000 replicates for the NJ tree. ClustalX 1.81 (Thompson et al 1997) was used to align the AM fungal sequences from this study along with 43 reference sequences from GenBank. The alignments were then manually adjusted using Bioedit Ver. 5.0.6 (Hall 2001). The tree was rooted with a non-flagellate protozoan *Corallochytrium limacisporum* sequence obtained from Genbank (Accession no. L42528).

Statistical Analysis. We tested whether the abundance of AMF sequence types differed between the *Clintonia* roots collected under the three different canopy types and between sites of different post-fire ages. For this analysis, data for each plot of similar canopy type of each fire year were pooled, which resulted in three replicates per group. The data were not normally distributed, therefore the Mann-Whitney U and the Kruskal-Wallis H tests were used (SPSS, version 10.1). Tests were performed only with groups which had a minimum of 5 clones per sequence group, therefore omitting comparisons among groups with rare sequence types.

Results

Vegetation analysis. Vegetation data for the overstory, shrub, and understory layers were averaged for all plots of similar canopy type and year (Fig. 1). In all 18 plots, the upper canopy was still dominated (>75%) by either trembling aspen, white birch or spruce-fir, as was originally recorded in 1994 (Fig. 1a). The most common species in the shrub layer were *Acer spicatum* Lam. an arbuscular mycorrhizal species, followed by *Abies balsamea* (L.) Mil., *Corylus cornuta* Marsh. and *Picea glauca* (Moench) Voss., all of which are colonized by ECM fungi (Fig. 1b). The most common understory plants included: *Aster macrophyllus* L., *Aralia naudicaulis* L., *Linnaea borealis L., Acer spicatum* seedlings, *Cornus canadensis* L., *Rubus pubescens* Raf. , *and Viola renifolia* A. Gray. The mean percent cover of AM plants *vs.* ECM plants in the understory for each canopy type per fire year is shown in Fig. 1c.

AM Colonization rates. The AM colonization rates for 31 of the 54 roots samples collected in this study were calculated. The morphology of the AMF colonizing the roots was similar to that published by Widden (1996). Fourteen plants from the 1870 site and seventeen plants from 1916 site were analyzed. Colonization rates per plot ranged from 42 to 78% (Table 1).

RFLP analysis, diversity, and sequence analysis. In total, 576 clones were screened. Six clones were eliminated from the analysis after the RFLP screening because band lengths did not add up to ~550bp. The remaining 570

clones were divided into 92 different RFLP types, all of which were sequenced. Fifty two of the sequences closely matched *Glomus* sequences. Of the 40 remaining sequences, one was closely related to a *Penicillium sp.*, another closely matched a bacterium and another had a sequence that matched *Clintonia borealis*. Seventeen other sequences did not match any other sequence in Genbank, and we did not obtain clear DNA sequences for the remaining 20. Thus all these sequences were excluded from the analysis. The 20 RFLP types that did not sequence very well only occurred once, thus we did not attempt to re-sequence them. 522 of the 576 clones screened belonged to one of the 52 RFLP types that had high sequence similarity (>90%) to *Glomus* spp.

Both the NJ and MP analyses of the 18S SSU sequences collected from the *Clintonia* roots revealed 10 similar clusters. As both methods gave similar results, only the NJ tree is shown in Fig. 2. The sequence identity within the clusters ranges from 97.3 to 100%. All 52 sequences have been submitted to the Genbank database (Accession nos. DQ122622 to DQ122673, Appendix 1). All sequences obtained from this study fall within the Glomeraceae. The QU-Glo1 group (Fig. 2) contains *Glomus* sequences collected from a variety of plants from different environments, as well as our most abundant sequence type. Sequences collected from legume and non-legume plants collected from a dune grassland in Holland, from *Pulsatilla* spp. in Estonia, from *Agrostis capillaris* L. and *Trifolium repens* L. from a grassland in Scotland and from *Hyancinthoides non-scripta* (L.) Chouard ex Rothm., *Gleochoma hederacea* L. and *Ajuga reptans* L. roots in a temperate forest in England all cluster within this group. Group QU-

Glo4 also contains sequences from the Estonian and Scottish studies mentioned above. Groups QU-Glo2 and QU-Glo9 contain sequences obtained from seedlings of indigenous woody plants in a warm-temperate deciduous broad-leaved forest in Japan. Group QU-Glo3 contains a sequence submitted by the University of York, and another submitted from the University of Montana from undisclosed host plants. Group QU-Glo5 also contains sequences collected from studies of dune grassland in Holland, from Estonia and from a grassland in Scotland (all of which were mentioned in relation to group QU-Glo1). This group also contains sequences from *Thymus polytrichus* A. Kerner ex Borbás ssp. *britannicus* (Ronn.) Kerguelen from metal contaminated soil in northern England and from *Phragmites australis* (Cav.) in a temperate wetland in Germany. Group QU-Glo6 contains sequences from the dune grassland in Holland and sequences submitted by the University of Kansas and the University of Montana from unknown host plants. Sequences in the QU-Glo7 group include 2 from *Phragmites australis* (Cav.) collected from a temperate wetland in Germany. Group QU-Glo8 contains sequences from *Thymus polytrichus* in metal contaminated soils in northern England. QU-Glo3 contains sequences from temperate grassland in the UK and one deposited in Genbank from the University of Missouri (US) and forms a clade with *G.intraradices.* QU-Glo10 included our sequence AMR31 and a known *Glomus* species, *Glomus viscosum.*

Richness and diversity indices based on the 10 AM sequence groups were calculated for each canopy type with the data from the 6 plots from the 2 fire years grouped together. The highest diversity was found in the birch plots,

with a Shannon's diversity index of 1.41, followed by the aspen and conifer plots with a diversity index of 1.03, and 0.95 (respectively). AM richness for the pooled plots of similar canopy type are 8, 6, and 8 for the birch, conifer, and aspen plots respectively.

The dominant sequence group, QU-Glo 1, represented 66.9% of the clones. This sequence was found in all canopy types, and it dominated 13 (>50% of clones analyzed) of the 18 plots. Sequence groups QU-Glo 2, 4, and 5 comprised 9.2, 7.9, and 6.5 % of the clones, respectively. Sequence groups QU-Glo 3, 8, and 9 had frequencies between 2-4%, and all other sequence groups constituted less than 1% of the clones (Fig. 3). Pair wise comparisons of the 39 sequenced clones of the most common RFLP type, showed that all sequences were ≥99% similar. Only one of those sequences, therefore, was used in the construction of the neighbour joining tree, sequence AMR1, which clusters in the QU-Glo1 group (Fig. 2).

Differences under the varying canopy types and between sites of different post fire ages. Most of the plots were dominated by the QU-Glo1 sequence group (Fig. 3). This sequence group was dominant in all plots except the three 1870 birch plots and the two 1916 aspen plots (Fig. 3). The statistical analyses did reveal that the abundance of this sequence group between the birch plots of the two post fire ages was significantly different ($p=0.05$), and the abundance of QU-Glo1 between the 1870 birch plots was also significantly different from that of the 1870 conifer and aspen plots ($p=0.05$), however the abundance of this sequence

type did not differ significantly between the 1870 conifer and aspen plots. There were no significant differences in the abundance of sequence groups among the plots of different canopy type from the 1916 site.

The three 1870 birch plots have a high percentage of AM plants in their understory and have an approximately 60% cover of mountain maple (an AM plant) in their shrub layer, which is higher than that found in any other plots (Fig. 1b and 1c). The abundance of mountain maple and AM herbs may be contributing to the lack of dominance by the QU-Glo1 sequence group in these three plots. However, two of the 1916 aspen plots also were not dominated by this group; although they did have a relatively high percentage of AM plants in the understory, they did not have a dense AM cover in the shrub layer (Fig. 1b and c).

Although the remaining AM groups were not found in high numbers, we saw that certain AM groups were found exclusively in certain plot types. QU-Glo 6 and 7 were exclusively found in the aspen plots and QU-Glo 10 was only present in a birch plot. QU-Glo 2 was only found in birch and conifer plots, and QU-Glo 9 was only found in birch and aspen plots.

Figure 1. Analysis of the (a) overstory, (b) shrub layer, (c) percent cover of AM vs. ECM species. The percent cover for each species, or group of species (Fig. 1c) is an average value of the three 100m^2 plots of similar age.

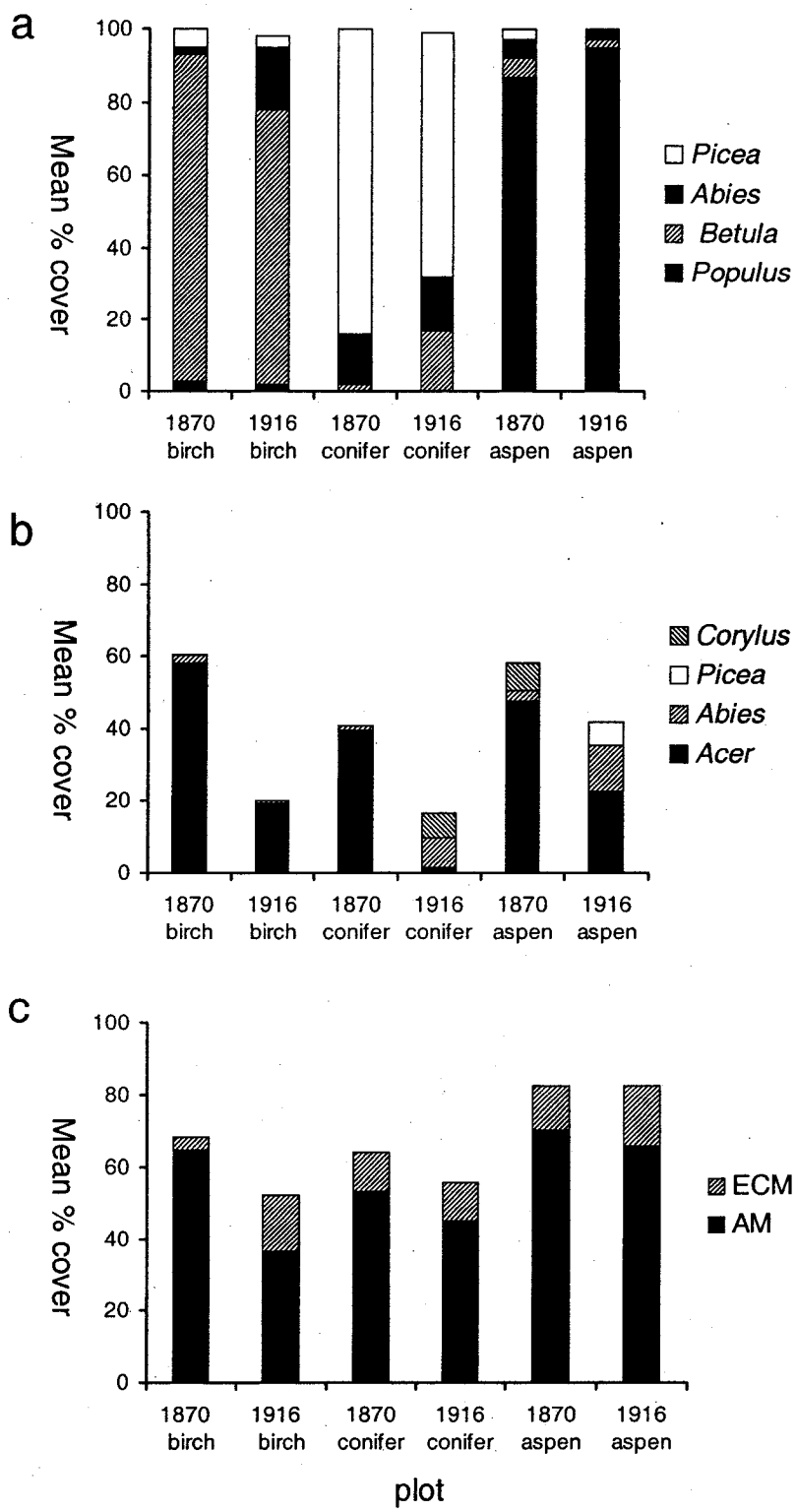
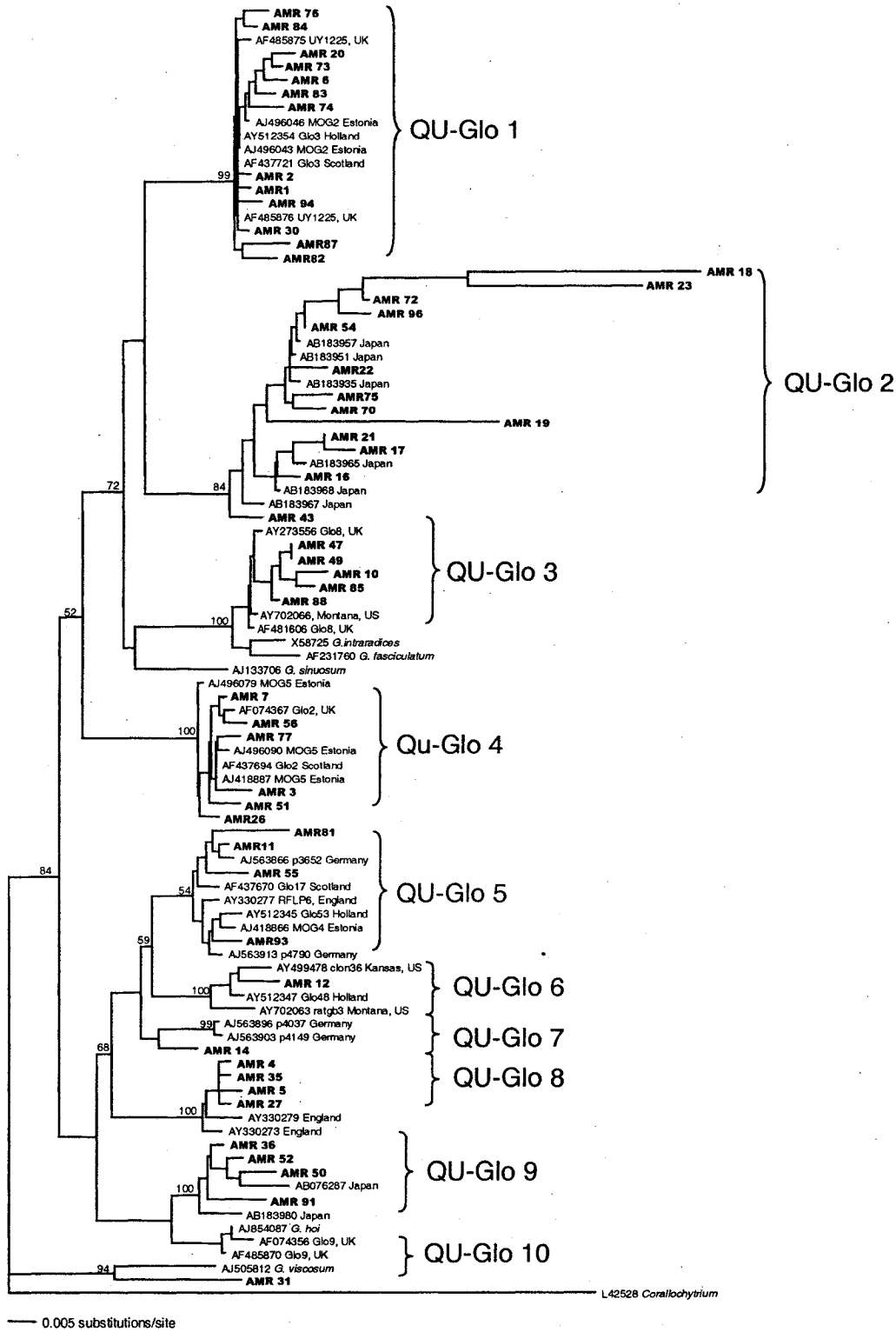

a
100
80
60
40
20
0
Mean % cover
Picea
Abies
Betula
Populus
1870 birch
1916 birch
1870 conifer
1916 conifer
1870 aspen
1916 aspen
b
100
80
60
40
20
0
Mean % cover
Corylus
Picea
Abies
Acer
1870 birch
1916 birch
1870 conifer
1916 conifer
1870 aspen
1916 aspen
c
100
80
60
40
20
0
Mean % cover
ECM
AM
1870 birch
1916 birch
1870 conifer
1916 conifer
1870 aspen
1916 aspen
plot

Figure 2. Partial 18S rRNA gene neighbour-joining tree demonstrating the placement of the sequenced RFLP types with 42 *Glomus* reference sequences obtained from Genbank. Sequences from Genbank are identified with their accession numbers, followed by a code name if available and geographic location from which sequence was obtained. Sequenced RFLP types are shown in bold, and are labeled as AMR followed by their RFLP number code. Bootstrap values (1000 replicates) are shown at the major nodes. The scale bar at the bottom left is proportional to branch length.


AMR 76
AMR 84
AF485875 UY1225, UK
AMR 20
AMR 73
AMR 6
AMR 83
AMR 74
AJ496046 MOG2 Estonia
AY512354 Glo3 Holland
AJ496043 MOG2 Estonia
AF437721 Glo3 Scotland
AMR 2
AMR1
AMR 94
AF485876 UY1225, UK
AMR 30
AMR87
AMR82
QU-Glo 1
AMR 18
AMR 23
AMR 72
AMR 96
AMR 54
AB183957 Japan
AB183951 Japan
AMR22
AB183935 Japan
AMR75
AMR 70
AMR 19
AMR 21
AMR 17
AB183965 Japan
AMR 16
AB183968 Japan
AB183967 Japan
AMR 43
QU-Glo 2
AY273556 Glo8, UK
AMR 47
AMR 49
AMR 10
AMR 85
AMR 88
AY702066, Montana, US
AF481606 Glo8, UK
X58725 G.intraradices
AF231760 G. fasciculatum
AJ133706 G. sinuosum
QU-Glo 3
AJ496079 MOG5 Estonia
AMR 7
AF074367 Glo2, UK
AMR 56
AMR 77
AJ496090 MOG5 Estonia
AF437694 Glo2 Scotland
AJ418887 MOG5 Estonia
AMR 3
AMR 51
AMR26
Qu-Glo 4
AMR81
AMR11
AJ563866 p3652 Germany
AMR 55
AF437670 Glo17 Scotland
AY330277 RFLP6, England
AY512345 Glo53 Holland
AJ418866 MOG4 Estonia
AMR93
AJ563913 p4790 Germany
QU-Glo 5
AY499478 clon36 Kansas, US
AMR 12
AY512347 Glo48 Holland
AY702063 ratgb3 Montana, US
AJ563896 p4037 Germany
AJ563903 p4149 Germany
AMR 14
AMR 4
AMR 35
AMR 5
AMR 27
AY330279 England
AY330273 England
QU-Glo 6
QU-Glo 7
QU-Glo 8
AMR 36
AMR 52
AMR 50
AB076287 Japan
AMR 91
AB183980 Japan
QU-Glo 9
AJ854087 G. hoi
AF074356 Glo9, UK
AF485870 Glo9, UK
QU-Glo 10
AJ505812 G. viscosum
AMR 31
L42528 Corallochytrium
0.005 substitutions/site

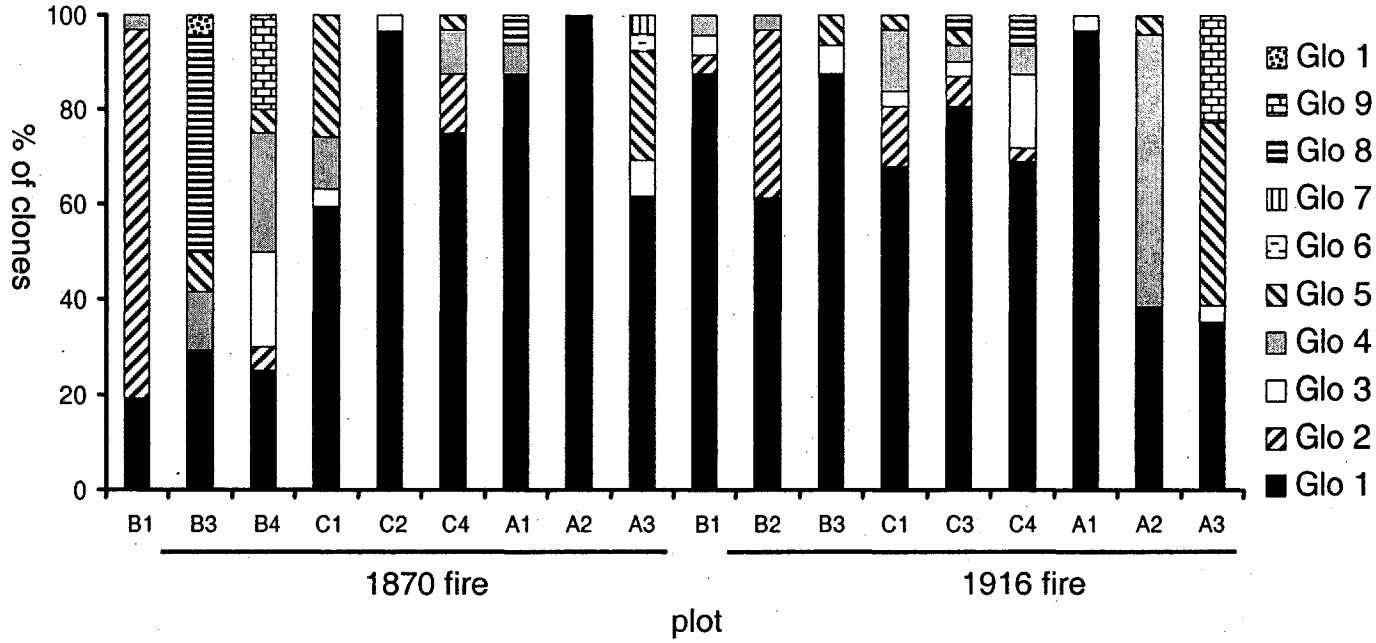


Figure 3. AMF communities in the roots of *Clintonia borealis*. AMF communities are represented as the percentage of clones per AMF sequence type. Each bar corresponds to one of the 18 plots examined. Bars are labeled by their canopy type (B for birch, C for conifer, and A for aspen plots), and the last number represents the plot series number.

Table 1. Arbuscular mycorrhizal colonization rates of some of the plants collected from the 18 plots.

Fire Year	Canopy type	Plot #	# Plants	mean % colonization/ plot	mean % colonization/ canopy type/fire year
1870	birch	1	2	63	
		3	3	71	
		4	3	85	73
	conifer	1	3	91	
		2	1	61	76
	aspen	1	1	70	
		2	1	74	72
1916	birch	1	3	40	
		2	1	55	
		3	2	30	42
	conifer	1	3	65	
		3	1	58	
		4	2	78	67
	aspen	1	2	66	
		2	1	99	
		3	2	68	78

Table 2. Comparison of AM richness and Shannon's diversity (H′) based on the SSU rRNA gene from 9 other studies of plant roots collected in natural ecosystems.

Ecosystem	N[a]	# Clones[b]	TR[c]	TH′[d]	H′[e]	R[f]
Woodland, N.Yorkshire, UK[g]	5	154	11	1.44	NA[u]	NA
Woodland, N.Yorkshire, UK[h]	1	141	8	1.67	winter: 1.62 summer: 1.36	6 7
Grassland, Scotland[i]	2	2001	24	1.71	NA	NA
Tropical forest, Panama[j]	1	558	18	2.28	3-mth old[q]: 1.96 1 yr old: 1.75 2 yr old: 1.71 5 yr old: 0.79	13 15 8 7
Tropical forest, Panama[k]	2	>1300	23	2.33	*Faramea* sp.: sample period 1: 1.53 sample period 2: 2.06 *Tetragastris* sp.: sample period 1: 1.98 sample period 2: 2.07	 16 16 14 15
Woodland, N. Yorshire, UK[l]	5	116	10	1.60	*Glechoma* (A[r]):1.16 *Ajuga* (A): 1.03 *Acer* (A): 1.24 *Epilobium* (Q[r]): 0.69 *Rubus* (Q): 1.20	5 5 4 2 6

Table 2, Continued.

Ecosystem	N[a]	# Clones[b]	TR[c]	TH'[d]	H'[e]	R[f]
Boreal forest & grassland, Estonia[m]	2	NA	6	1.33	*P.pratensis*[s], 2(G1):1.04	3
					1(F1): 0.00	1
					3(B1): 0.56	2
					1(G2): 0.00	1
					P. patens[s], 1(F1): 0.00	1
					2(B1): 0.64	2
					1(F2): 0.00	1
					1(B2): 0.00	1
Dune grassland, Holland[n]	6	562	15	NA	*Festuca*: 1.43	11
					Plantago: 1.43	11
					Hieracium: 0.86	4
					Lotus: 1.40	10
					Trifolium: 1.20	6
					Ononis: 1.25	5
Temperate Wetland, Germany[o]	1	546	21	2.4	site A: 1.2 - 1.7[t]	6 - 9
					site B: 1.5 - 2.1[t]	7 - 10
Mixed-wood boreal forest, Canada[p]	1	522	10	1.22	birch: 1.41	8
					conifer: 0.99	6
					aspen: 1.03	8

[a]number of host species analyzed, [b]total number of clones screened, [c]total richness based on all plant species and/or from all sampling sites as presented in original paper, [d]total diversity based on all plant species and/or from all sampling sites as presented in original paper, [e]diversity, recalculated on the basis of single plant species and/or from a single sampling period, [f]richness, recalculated on the basis of single plant species and/or from a single sampling period

[g]Helgason et al (1998), [h]Helgason et al (1999), [i]Vanderkoornhuyse et al (2002), [j]Husband et al (2002a), [k]Husband et al (2002b), [l]Helgason et al (2002), [m]Opik et al (2003), [n]Scheublin et al (2004), [o]Wirsel (2004), [p]present study.

[q]Collected from same cohort, over a 3-yr period, for the last sampling period selected plants of 2 age groups.

[r]Collected plants under two canopy types: *Acer* (A), *Quercus* (Q), with two sampling plots under each.

[s]*P. pratensis* = *Pulsatilla pratensis*, *P. patens* = *Pulsatilla patens*. The number following the plant name represents the number of root samples that were successfully amplified. The following letter and number code in brackets represents the sampling sites: G1 and G2 = dry meadow, F1 and F2= boreal pine forest, B1= sandy area bordered by pine forest, B2= roadside bordered by pine forest.

[t]Range of diversity and richness values for all sampling periods.

[u]NA= data not available

Discussion

The data presented in this paper are the first look at AM sequences in plant roots collected from a boreal mixed-wood forest in eastern Canada. Our main findings are that there was a high dominance by one AM group and that there were no significant differences in the abundance of AM sequence groups among the different stand types. All sequences collected from the *Clintonia* roots belong to one family, the Glomeraceae. Also, most of the sequences present in the *Clintonia* roots do not match sequences of known cultures of AM fungi but they did match other uncultured AM sequences obtained from plant roots collected from various field/natural sites.

Dominance by *Glomus* spp. in plant roots has been reported from a number of different habitats ranging from stable forest environments (Husband et al 2002a, and 2002b), wetlands (Wirsel 2004), dune grasslands (Scheublin et al 2004), to highly disturbed agricultural fields (Daniell et al 2001). The boreal mixed-wood forest is a stable environment that is dominated by ECM trees. Thus, it may be very beneficial for an understory herbaceous plant growing under a tree canopy to tap into the already established AM mycelial network and quickly gain access to the underground nutrient absorbing system. As it is known that different AM fungi can affect plants in different ways (Klironomos 2003, van der Heijden et al 2003), a high diversity of AM fungi may be needed for seedlings at the critical establishment stage but once established in a fairly stable environment, AM fungi that are best suited to that particular stable environment may be selected for. Husband et al (2002a, 2002b) reported a decline in fungal diversity and

evenness and noted a gradual replacement of fungal types over time in the AM fungi colonizing *Tetragastris* and *Faramea* seedlings collected from a tropical forest. In *C. borealis,* new ramets arise from rhizomes, rhizome sections can persist for 10 or more years and the establishment of new patches is rare (Pitelka et al 1985). In this situation, AMF that can quickly colonize available rootlets via hyphae may be selected for and these selection pressures may result in an environment with low AMF diversity due to the dominance of the fungi with a high preference for *Clintonia borealis.* Species within the Glomeraceae may be best suited to such an environment as studies have shown that they can colonize new roots from hyphal fragments (Tommerup and Abbott 1981) and can do so at a much faster rate than members of the Acaulosporaceae and Gigasporaceae (Hart and Reader 2002). A new *C. borealis* plant developing along an already existing rhizome does not experience the same establishment pressures that a seedling may encounter and may simply benefit the most by quickly tapping into the already established mycelium.

The dominant sequence group, QU-Glo 1 (Fig. 2) contains AM sequences from a temperate deciduous woodland in England (AF485876), from *Pulsatilla* plants from Estonia (AJ496046), and from a dune grassland in Holland (AY512354). This group seems to represent a generalist fungus with a broad host and geographical range. In fact, most of the sequences found in this study cluster with other *Glomus* sequences from a variety of environments. The primer pair AM1 and NS31, used in all of these studies may be contributing to the marked similarities in sequences obtained from studies from distant geographical

locations. The AM1 primer was designed on the basis of only 12 AMF sequences (Helgason et al 1999) and it is known that it can amplify AM sequences from only 3 families within the Glomeromycota (the *Glomeraceae*, *Gigasporaceae* and *Acaulosporacae*) (Daniell et al 2001)). Furthermore, de Souza et al (2004) have discovered that the V3-V4 region of the 18S rRNA gene, the region amplified by AM1 and NS31, did not contain enough variation to discriminate between different *Gigaspora* species. However, based on studies of spores found in the soil, species of *Glomus* are by far the most abundant AM fungi in the soils of the Eastern Canadian Shield, with some *Acaulospora* spp. also being reported (Klironomos 1995, Moutoglis and Widden 1996). Nevertheless, as sampling was done just once and the primers may exhibit amplification bias, the 10 different sequences obtained may not be an entirely accurate representation of the AMF community in this ecosystem.

The AM richness and diversity from this boreal forest was higher than that observed in an arable field (Daniell et al 2001), but was lower than that seen in many other studies from a range of habitats (see Table 2). However in most of the studies listed in Table 2, more than one plant host was analysed and plants were sampled at more than one time, both of which can have a great impact on AMF richness and diversity (Helgason et al 2002). In order for our data to be more comparable with the data from these other studies, diversity and richness based on single plant host from a single sampling period or site were calculated and are presented in the last two columns of table 2. When the data is shown in such a manner, we see that the diversity observed from the boreal mixed-wood

forest is lower than that observed in a tropical forest (Husband et al 2002a, 2002b) and a temperate wetland in Germany (Wirsel 2004), but similar to that seen in a dune grassland in Holland (Scheublin et al 2004). The diversity in this study is similar to that of a woodland in North Yorkshire, UK (Helgason et al 2002), but the number of clones screened in that study was much lower than those screened in the present study (see table 2), therefore the values from the 2 studies are not very comparable.

Another objective of this study was to see whether there would be any shifts in the AMF community in plants collected under the different canopy types. The most common sequence group QU-Glo1 was present in significantly lower amounts in the 1870 birch plots compared to the other canopy types of similar age, but there were no differences in the abundance of QU-Glo1 between plots of different canopy type from the 1916 sites, hence this finding could not be attributed to difference in tree composition. Although Clintonia plants were collected under an ECM canopy, in each plot a lush AM herbaceous layer was present and in many plots a shrub layer consisting mainly of Acer was present (Fig. 1). In the Helgason et al (1999) study, differences in the types of AM sequences found in the *Hyancinthoides* roots collected under either a sycamore or oak dominated canopy were seen. Differences in the types of fungi colonizing the roots of *Clintonia* in the different plot types were not observed, but all plot types in this study were dominated by ECM tree species while the *Hyancinthoides* were collected under a an ECM species (oak) and an AM plant (sycamore). The only other study where an AM herbaceous plant was collected

from under an ECM canopy is that of Opik et al (2003) who looked at the AM community in the roots of 2 *Pulsatilla* species that were collected from a Scots Pine forest Estonia. In that study however, only 6 root samples collected from the forest or roadsides bordered by pine forest were successfully amplified. The resulting diversity and richness values were quite low, probably due to the small sample size.

Most of the main findings of this study are in agreement with those of other studies using similar methods. We have seen that most of the AM sequence groups found do not match sequences from known cultures but closely match other sequences obtained from roots collected in the field. Thus further emphasizes the lack of knowledge of these organisms in natural environments. We have shown that *Glomus* spp. dominate in this forest system, as was seen in several other studies from a variety of environments. Contrary to Helgason et al. (1999), we did not see any differences in the distribution of the different AM taxa in *Clintonia* plants collected from under the different canopy types. What is of particular interest is that the sequences found in this study are very similar to those found in different plant hosts from a variety of ecosystems from many parts of the world. The fact that the most common sequence type found in this boreal forest is similar to AM sequences found from a wide variety of plants from various geographic locations may indicate that the AM fungi have some sort of evolutionary mechanism that may be quite particular to this group of symbiotic organisms or these similarities may be due to primer biases and we are amplifying only a small portion of the actual AMF colonizing plant roots. More

field-based studies targeting several AM genes are needed to obtain a better understanding of AMF. We conclude by agreeing with Rodriguez's et al (2004) plea that future work on the diversity of the AMF should include data on a few genes, in order to further expand our knowledge on the ecology and genetics of these organisms.

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Chapter 3: Plant community influences on soil microfungal assemblages in boreal-mixed-wood forests.

Abstract

We studied the relationships between assemblages of soil microfungi and plant communities in the southern boreal mixed-wood forests of Québec. Sampling took place in eighteen 100m^2 plots from an existing research site. Plots were separated into 3 categories based on dominant overstory tree species: i) trembling aspen, ii) white birch, and iii) a mixture of white spruce and balsam fir. Within each plot, a 1m^2 sub-plot was established in which the understory herbaceous layer was surveyed and soil cores were collected. Microfungi were isolated from soil cores using the soil washing technique and isolates were identified morphologically. To support our morphological identifications, DNA sequences were also obtained for the most abundant microfungi. The most frequently occurring microfungal species were *Penicillium thomii*, *P.spinulosum*, *P. janthinellum*, *Penicillium* sp., *P. melinii*, *Trichoderma polysporum*, *T. viride*, *T. hamatum*, *Mortierella ramanniana*, *Geomyces pannorum*, *Cylindrocarpon didymum*, *Mortierella* sp. and *Mucor hiemalis*. Multivariate analyses (redundancy analysis followed by variance partitioning) revealed that most of the variation in microfungal communities was explained by understory plant species composition, as opposed to soil chemistry or overstory tree species. In this floristically diverse system, saprophytic microfungal assemblages were not correlated with the overstory tree species, but were significantly correlated with

the main understory herbs, thereby reflecting differences at a smaller spatial scale.

Introduction

Fungi are one of the most important functional groups of soil microbes, and are critical to nutrient cycling, transport of nutrients to plants, plant growth and disease suppression (Christensen 1989, Thorn 1997). Despite their well documented role in ecosystem functioning, it is estimated that only 5% of fungal species have been described (Hawksworth 2001), and little is known about their dynamics, community structure and diversity.

Soil organisms rely mainly on carbon from root exudates (Grayston et al 1996) and litter inputs (Conn and Dighton 2000, Wardle 2002a,b) for growth; therefore the chemical composition of these substrates exerts a large influence on soil fungal communities (Frankland 1966, Christensen 1969, Lumley et al 2001). Past studies have also revealed that plant species diversity influences fungal community structure. Apinis (1972) stated that fungal communities reflect the conditions of the soil environment and of the accompanying plant community. Christensen (1981) compared 33 microfungal communities from several different environments and uncovered a clear correspondence between microfungal species composition and vegetation type, and concluded that soil microfungi are remarkable indicators of environmental similarity. Widden (1986) observed differences in microfungal assemblages between coniferous and deciduous forests in southern Québec. McLean and Hutha (2002) reported differences in microfungal assemblages collected under birch, spruce and in arable fields and

stated that the differences between fungal communities are primarily attributable to differences in litter quality. Similarly, a microfungal community analysis of alpine soils by Bisset and Parkinson (1979) indicated that the major source of variation in microfungal species composition is attributable to differences among sites which are largely determined by vegetation.

The mixed boreal forest of eastern Canada is an ecosystem in which the ecological processes are controlled by disturbances such as fire and pest outbreaks, which results in a heterogeneous landscape of different stand types of differing ages (Bergeron 2000). At the Lac Duparquet Research and Teaching Forest in Abitibi Québec, long term plots with differing proportions of balsam fir (*Abies balsamea* (L.) Mill.), white spruce (*Picea glauca* (Moench) Voss), trembling aspen (*Populus tremuloides* Michx.), and paper birch (*Betula papyrifera* Marsh.) have been established. As soil chemical analyses had already been conducted in a previous study (Legaré et al 2001), we selected plots situated on similar clay deposits which had similar chemical properties in order to focus on the effects of the plant community on fungal communities.

The present study is part of a larger project on the relationships between plant and microbial communities in the boreal mixed-wood forests of Québec. We have previously focused on the ectomycorrhizal (EM) fungal communities on this site and found strong positive correlations between overstory tree composition and diversity and EM fungal species composition and diversity (Kernaghan et al 2003, DeBellis et al 2006). The present study was undertaken to further characterize the fungal communities on this site, by examining the soil

microfungal assemblages and their relationships with overstory tree species composition and understory species composition.

As the EM fungi form a symbiotic relationship with their plant hosts, correlations between these fungi and the abovegroundcanopy trees was expected. However, as the soil microfungi are mainly litter-decomposers, they are likely to be more affected by small scale heterogeneity in the soil which is determined by litter inputs from plants in close proximity. We therefore hypothesized that, unlike the EM fungi, the assemblages of microfungi would be more strongly correlated with the immediately surrounding understory vegetation than with the abovegroundcanopy trees.

Materials and Methods

Site description. The study area is located in the Lac Duparquet Research and Teaching Forest, in northwestern Québec (48°30′N, 79°20′W). This area is part of the western balsam fir – paper birch bio-climatic domain (Grondin 1996), which extends over the Clay Belt region of Québec and Ontario. The closest weather station to the study area is located at La Sarre, 35 km north of Lac Duparquet. The average annual temperature is 0.8°C, daily mean temperature is –17.9°C for January and 16.8°C for July, and the average annual precipitation totals 856.8 mm (Environment Canada 1993). By dendrochronological analysis, Bergeron (1991) and Dansereau and Bergeron (1993) determined that the stands used in the present study originated from fires that took place 82 to 135 years ago. In the early stages of succession, paper birch, trembling aspen, or jack pine (*Pinus banksiana* Lamb.), dominate the forest. If stands are not subjected to any major

disturbances, they become dominated by balsam fir and white cedar (*Thuja occidentalis* L.) (Legaré et al 2001).

Sampling design. Soil cores were collected from forests that originated either from a fire in 1870 (1870 fire) or from fires in 1916 or 1923 (1916/1923 fires). Half of the cores were collected from plots from the 1870 fire and half from the 1916/23 fires, (hereafter referred to as the 1916 plots). Within both the 1870 and 1916/1923 sites, three replicate plots (100 m^2) of three different canopy types; (i) trembling aspen dominated, (ii) white birch dominated and (iii) white spruce - balsam fir dominated, were selected. Sampling took place in a total of 18 plots (2 sites × 3 canopy types × 3 replicate plots). A plot was assigned to one of the 3 categories when the corresponding species or group of species exceeded 75% of the total basal area of that plot. In all plots, dominant trees originated after fire, except for the 1870 aspen plots, which are a second cohort of aspen (Bergeron 1991). All plots were selected from an existing design which was initially set up in 1994 (Legaré et al 2001). In August 2002, we re-analyzed the overstory composition of these plots to ensure that the data still reflected the overstory composition recorded in 1994. The upper canopy in each plot was still dominated (>75%) by either trembling aspen, white birch and spruce-fir, as recorded by Legaré et al (2001), but we also noted a lower canopy layer of ~2 meters in most plots. The percent cover of each tree species in this lower canopy was recorded for each plot by visual observation. Due to the extreme heterogeneity of the understory vegetation, a 1m^2 sub-plot was arbitrarily marked at the southwest

corner within each 100m^2 plot and the percent cover of each understory plant (<1m in height, including tree seedlings) was estimated. Plants >1m in height were considered as the lower canopy. In the field, when the 1m^2 plot was established, all understory plants were identified and by visual observation percent cover was recorded. Also a digital photo of each 1m^2 plot was taken. At a later date the % cover and plant identifications were compared to the digital photos to ensure the data collected in the field was accurate. The nomenclature for plant species follows Marie-Victorin (1995).

Organic soil analyses. For the soil chemical analyses, data was taken from a previous study in which 4 samples were taken from the FH horizon, pooled within plots, air dried, then ground prior to analyses for pH, total Ca, K, Mn, Mg, P, N, and organic carbon (Legaré et al 2001).

Sampling, Isolation and identification of fungi. In August 2004, a core of organic soil (~10cm in depth) was taken with a 7.5 cm diameter corer from each of the 18 1m^2 sub-plots described above. Soil cores were placed in Ziploc® (S. C. Johnson & Son, Inc) bags, stored in a cooler with ice packs and transported to the laboratory. In the laboratory, cores were stored at 4°C. Within a week of sampling, the soil microfungi were enumerated using the soil washing method (Parkinson and Williams 1961). Five-gram sub samples from each soil core were washed using twenty 1 min washings in an apparatus similar to that described by Bissett and Widden (1972). One hundred soil particles from each sample were

plated onto Czapek-Dox agar (Oxoid Ltd., Code CM97) acidified to pH 4.5 with lactic acid. Plates were incubated at 15°C for 10 days and each fungal colony was morphologically identified and enumerated. Colonies that were not readily identified were sub-cultured onto 2% malt extract agar (Oxoid Ltd., Code LP0039) for future identification. Sporulating microfungi were identified using keys to the soil microfungi including Domsch et al (1980), Barron (1968), and Booth (1966). To identify the *Penicillium* isolates to species, the methods and key provided by Pitt (1979) were followed. Some isolates however, could not be identified to species and were given a code number.

Molecular analysis of the microfungi. DNA was extracted from the 25 most common microfungi. Isolates were grown on 2% malt agar for 2 weeks. Mycelia were then removed and the DNA extracted using a modification of the protocol outlined by Gardes and Bruns (1993). Tissue was ground in liquid nitrogen in a ceramic mortar and incubated for 1 h at 65°C in 600 μl 2X CTAB extraction buffer. Six hundred μl of chloroform: isoamyl alcohol (24:1) were then added and the mixture was centrifuged at 16 000 g for 15 min. The supernatant was then mixed with 600 μl isopropanol and again centrifuged at 16 000 g for 15 min. The resulting pellet was washed twice with 80% ethanol, air dried, and re-suspended in 50 μl water. The ITS1-5.8S-1TS2 region of the ribosomal DNA was amplified using the fungal specific primers ITS-1F (Gardes and Bruns 1993) and ITS4 (White et al 1990). The reactions were carried out in a final volume of 50 μl and included 0.2 mM dNTPs, 25 pmol of each primer, 2.5mM $MgCl_2$ and 2.5 units of Taq DNA polymerase. The thermal parameters used were similar to those cited

in Gardes and Bruns (1993). PCR products were sequenced at the at the McGill University and Genome Québec Innovation Centre using an ABI PRISM® 3730XL DNA Analyzer system with the ITS-1F primer. Sequence data were run through the BLAST search program in the Genbank database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/) to obtain the most similar sequences from the database.

Statistical Analysis. Relationships between overstory tree composition, understory vegetation, soil chemistry and the microfungal species composition were assessed using redundancy analysis (RDA). First, a detrended canonical correspondence analysis with detrending by segments was performed to obtain the gradient length of the taxa in the environmental space. As the taxa gradient was < 2 standard deviations, a linear response was assumed and RDA was selected as the analysis method (Leps and Smilauer 2003). RDA is a form of direct gradient analysis (ter Braak and Prentice 1988) that describes the variation between two multivariate data sets. More specifically, in this case a matrix of explanatory variables (% cover of each overstory tree, understory vegetation or soil chemistry) was used to quantify the variation in a matrix of response variables (microfungal community matrix using count data). In each analysis, step-wise forward selection (a test which is analogous to forward stepwise regression), was used to reduce the environmental variables to those most correlated with the axes. The variables and axes were tested for significance using a Monte-Carlo permutation test with 999 permutations. The RDAs were

carried out using the computer package CANOCO 4.5 (ter Braak and Smilauer 2002).

Variance partitioning. RDA with the microfungal species data and all 3 environmental data matrices revealed that the understory vegetation and soil chemistry explained a significant portion of the variance of the microfungal community. Hence these two environmental data matrices were analyzed together and the variance partitioning procedure (Borcard et al 1992) was used to further analyze the data, by providing a quantitative partitioning of the variance in the microfungal community data with the two environmental data matrices. The variance portioning decomposed the total variability in the microfungal data into 4 parts, (i) variation solely due to soil chemistry, (ii) variation solely due to understory plants, (iii) shared variation of the 2 environmental matrices, (iv) unexplained variation. To calculate the individual parts of the variability, the microfungal species data matrix and the understory and soil chemistry data matrices were subjected to a series of partially-constrained ordinations. The significance of each partial RDA was evaluated using the Monte Carlo permutation test with 999 permutations. All analyses were carried out using the computer package CANOCO 4.5 (ter Braak and Smilauer 2002).

Results

Understory plant community. A total of 42 different understory plants were found in the $1m^2$ plots. To investigate relationships between the microfungal

community and the understory community, plants present <5% were first removed from the analysis. The remaining plants were used in the RDA, with forward selection, which revealed that *Rubus pubescens* Raf, *Corylus cornuta* Marsh., *Abies balsamea* (L.) Mill, *and Aralia nudicaulis* L., were the understory plants which accounted for most of the variation in the microfungal species data. Table 1 shows the understory species richness in each plot, as well as the % cover of each plant used in the RDA including *Aster macrophyllus* L., as it was abundant in several plots.

Fungal community composition and species richness. A total of 74 different sporulating microfungi were isolated from the 18 plots. Five percent of the isolates did not sporulate and were grouped into a sterile category and remained unclassified. Microfungal species richness was 44, 43 and 48, for the birch, conifer and aspen dominated plots respectively. *Penicillium thomii*, *Trichoderma polysporum*, *Trichoderma viride*, *Penicillium spinulosum*, *Mortierella ramanniana* and *Penicillium janthinellum* were the most common microfungal species, colonizing >6% of the particles analyzed (Table 2). Along with the microfungi mentioned above, *Trichoderma hamatum*, *Geomyces pannorum*, *Penicillium* sp. #1, *Penicillium melinii*, *Cylindrocarpon didymum*, *Mortierella* sp. #5 and *Mucor hiemalis* were also fairly common, being present in more than 50% of the plots (Table 2).

Microfungal isolates were identified using morphological methods and the most common isolates were also characterized using molecular characters. DNA

sequences were obtained for the 25 most common microfungi in order to confirm our morphological identifications. Sequences were approximately 550 bp in length, with the exception of the sequence obtained for *Verticillium* sp. #10 which was only 180 bp. All sequences except for the one obtained for *Verticillium* sp. #10 have been submitted to the Genbank database and all were compared to those in Genbank using BLAST to obtain closely matching sequences (Table 3). Of these 25 microfungi, 18 were identified to species and 7 were identified to genus based on the morphological analysis (Table 3). Based on the BLAST searches in Genbank, 24 sequences were in agreement at the genus level with our morphological identifications of the fungi. Of the 18 isolates identified to species using morphological methods, 13 had species level matches with the sequence data. However, in some cases such as *Trichoderma polysporum* and the 2 *Cylindrocarpon* species, several species in Genbank had 99-98% sequence similarity with our sequence. In such cases, the sequence that matched our morphological identification was selected. In the remaining sequences, those obtained for *M. ramanniana, P. janczewski* and *P. janthinellum* had the highest sequence similarity to other Genbank sequences that were solely identified to the genus level (Table 3). For these sequences, we also performed reverse searches. We searched Genbank for sequences identified as these 3 species and the sequences were then compared. In the case of *P. janczewski* there was 91% sequence similarity with an identified Genbank sequence, *P. janthinellum* had a 95% sequence similarity to other *P. janthinellum* sequences, and *M. ramanniana* had a 96% sequence similarity in the 5.8S region to a *M.*

ramanniana isolate in Genbank. Our *M. ramanniana* sequence had 2 introns in the ITS 1 region that were not present in the Genbank sequences and this resulted in a lowered sequence identity for the whole length of the sequence. Four isolates not identified to species using morphological methods, *Mortierella* sp. #5, *Penicillium* sp. #1, and *Verticillium* spp. #2 and 6, had a 99% sequence identity match with *Mortierella humilis*, *Penicillium kojigenum*, *Verticillium suchlasporium, and Verticillium bulbillosum* respectively, and *Penicillium* sp. #3 has a 96% sequence similarity to *P. lividum.* Although some of our sequences that had not been identified to species did have a 99% similarity to other identified Genbank sequences, we chose not to adopt these species names as we have noted that some of our isolates that had been identified to species had more than one species in Genbank with 99-98% sequence similarity, and the reverse also occurred with some of the isolates that we had identified morphologically to species not having high sequence matches (≥ 96%) with similarly identified species in Genbank.

The closest match to *Chrysosporium merdarium* was an unidentified ascomycete, and it also had a very high sequence similarity to other Genbank sequences identified as either *Geomyces pannorum* or *Pseudogymnoascus roseus.* We do not think our identified *C. merdarium* is *G. pannorum* as we also isolated *G. pannorum*, and it was clearly distinguishable from *C. merdarium* based on morphological features. Although we obtained a short sequence for *Verticillium* sp. #10, its closest match from the Genbank database was a *Verticillium* species. Our isolates of *Trichoderma hamatum* (based on

morphological identification) had a 95% sequence similarity to *T. oblongisporum* (Table 3). These 2 species have very similar morphological traits and would have been regarded by Rifai (1969) as members of the *T. hamatum* species aggregate which was later partitioned into a number of species by Bissett (1991).

Microfungal community and upper and lower canopy cover. The microfungi included in these analyses were those found in 5 or more plots, thus 25 microfungi were included in the analysis. This reduced set of 25 isolates accounted for 89.2% of the total abundance. Redundancy analysis of the microfungal community dataset and the upper and lower canopy data matrix with step-wise forward selection showed that none of the variables were significantly related to the fungal community data, however, the variable which contributed the most to the species variation was percent overstory aspen cover (P=0.12). The resulting RDA with % aspen cover as the only variable in the environmental matrix, revealed that it was not significantly correlated with the microfungal species composition (P =0.15), explaining only 8.4% of the variability in the species data.

Microfungal community and soil chemistry. Two of the nine variables in the soil chemistry matrix; total carbon (P =0.008) and total calcium (P =0.024), were selected using the forward selection procedure. The RDA produced an ordination in which the first axis was significant (P =0.005), and the test of significance for all canonical axes was also significant (P =0.003). The

eigenvalues for the two first axes were 0.16, 0.07, and the species- environment correlations for the two first axes were 0.86, and 0.70. The first two axes of the ordination explained 23% of the variance, and most of this, 16%, was contributed by the first axis.

Microfungal community and understory vegetation. The forward selection procedure showed that *Rubus pubescens, Corylus cornuta, Abies balsamea, and Aralia nudicaulis*, were the understory plants which accounted for most of the variation in the microfungal species data. RDA with these four species produced an ordination in which the first and all canonical axes were significant (P=0.001, P =0.001, respectively). The eigenvalues for the three first axes were 0.22, 0.13, and 0.05, and the species- environment correlations for the three first axes were 0.96, 0.84, and 0.71. The first three axes of the ordination explain 39% of the variance, of which 22% was contributed by the first axis and another 12.5% was contributed by the second. The intra-set correlations between the environmental variables were examined to determine those environmental variables most correlated with each of the axes of the RDA. The first RDA axis, which accounted for most of the variation, was correlated with % *Rubus* and % *Corylus*. In the RDA bi plot (Fig. 1), we see a separation along the first axis with the 2 plots with the highest amount of *Corylus* (intra-set correlation -0.42) towards the negative direction of the first axis and the 2 plots with the most *Rubus* falling towards the positive (intra-set correlation 0.85). The second axis was most correlated with % *Abies* and % *Aralia*. Along the second axis, plots with *Abies*

and no *Aralia* (1870 conifer replicate #1, and 1916 birch replicate #1, Fig. 1) are in the positive direction of axis 2, with *Abies* having an intra-set correlation of 0.71 with axis 2, while the 1916 aspen plot replicate #1 is closer to the center as it has both *Aralia* and *Abies.* *Aralia* was the understory plant that explained most of the variation in the plots in the negative direction of Axis 2, with an intra-set correlation of -0.31. In Fig. 1, the plots with the highest amount of *Aralia* fall towards the negative direction of axis 2. Most of the other plots in the negative direction of axis 2 consisted of plots dominated by *Aster macrophyllus* L. or mixes of *Aster* and *Aralia.*

Variance partitioning. From the above mentioned RDAs, we obtained the total amount of variation in the fungal species data explained by the soil chemistry (23%) and understory data matrices (39%). By partitioning the variation in fungal species using partial RDA with the understory and soil chemistry matrices, we found that the variation was best explained by understory species, explaining 27.5% of the variation, and that soil chemistry accounted for only 10% of the variation. When the portion of the variation in the fungal species data for each individual component (i.e., understory and soil chemistry) was tested for significance, only the portion explained by the understory remains significant (understory vegetation P=0.037, soil chemistry P=0.351). Unexplained variation accounted for 49.5% of the total variation.

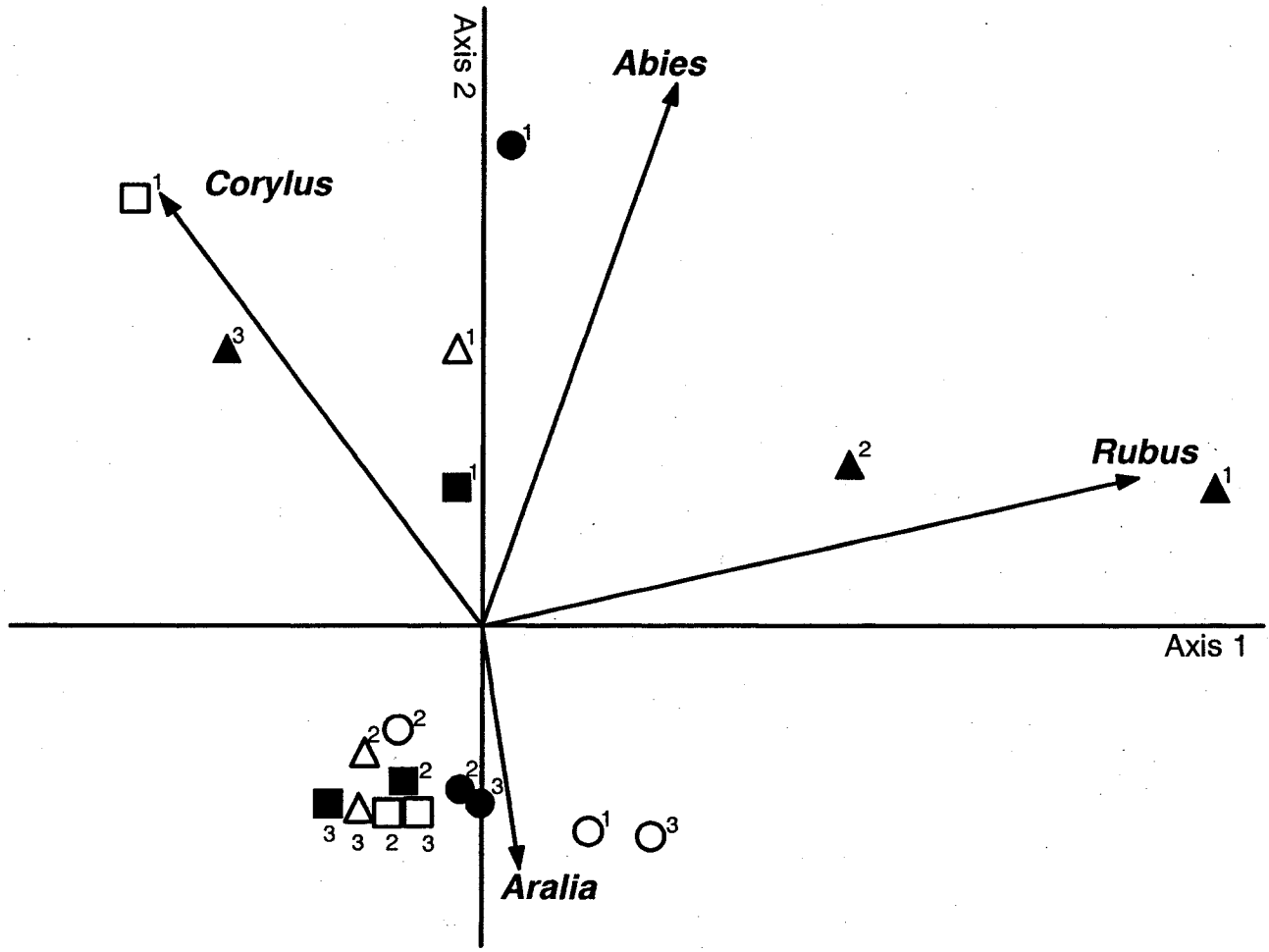
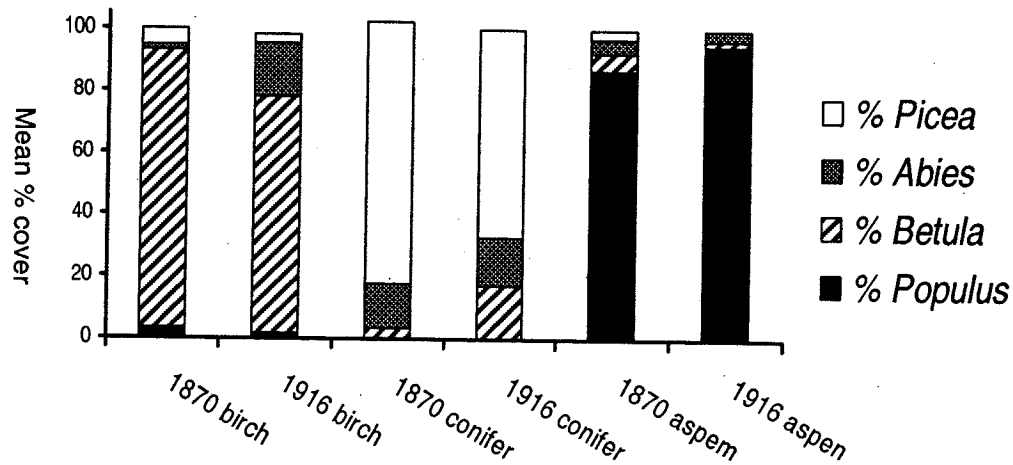


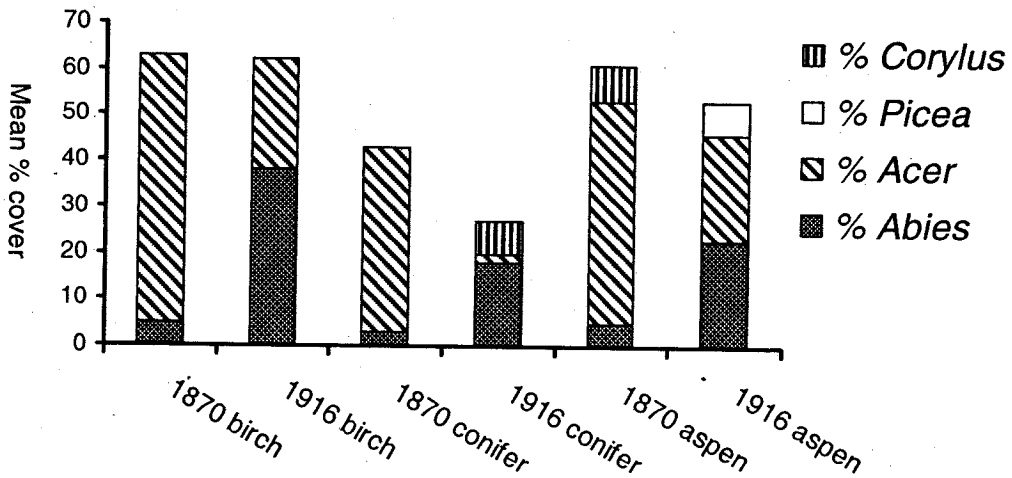
Figure 1. Redundancy analysis of the microfungal community (based on the 25 most frequent species) and understory vegetation from the 18 plots. Circles represent the aspen plots, triangles represent the conifer plots and the squares represent the birch plots. Open symbols represent plots from the 1870 fire, while the black symbols represent plots from the 1916 fire. Superscript numbers by each symbol correspond to the plot replicate number.

Figure 2. Analysis of the (a) overstory, and (b) lower canopy for each plot type. The percent cover for each species is an average value of the three $100m^2$ plots from the 1870 fire and the 1916 fire sites. Figure legend shows the genus names for each of the following species: *Picea glauca, Abies balsamea, Betula papyrifera, Populus tremuloides, Corylus cornuta,* and *Acer spicatum.*

a


Mean % cover
100
80
60
40
20
0
1870 birch
1916 birch
1870 conifer
1916 conifer
1870 aspem
1916 aspen
% Picea
% Abies
% Betula
% Populus

b


Mean % cover
70
60
50
40
30
20
10
0
1870 birch
1916 birch
1870 conifer
1916 conifer
1870 aspen
1916 aspen
% Corylus
% Picea
% Acer
% Abies

plot

Table 1. Understory richness (R) and percent cover of the main understory plants present in the eighteen 1m^2 plots.

Fire year	Plot type	plot #	Understory species (% cover)						Understory R
			Aralia	*Aster*	*Corylus*	*Rubus*	*Cornus*	*Abies*	
1870	Birch	1	30	5	20	0	0	0	9
		2	10	0	0	0	0	0	8
		3	10	50	0	0	5	0	7
	Conifer	1	0	0	0	0	0	35	7
		2	15	30	5	0	0	0	9
		3	5	80	0	0	0	0	7
	Aspen	1	75	15	0	0	0	0	6
		2	25	25	5	0	0	0	13
		3	60	30	0	0	0	0	10
1916	Birch	1	0	15	0	0	30	50	5
		2	10	30	0	5	30	0	10
		3	5	0	0	5	0	0	6
	Conifer	1	0	0	0	30	0	15	6
		2	10	0	5	20	20	10	9
		3	0	0	15	5	15	0	10
	Aspen	1	15	40	5	0	0	20	11
		2	20	20	5	0	0	20	6
		3	0	95	0	0	0	0	6

Table 2. Percentage of soil particles colonized and percent frequency of occurrence by each of the most common microfungal species isolated from the 18 plots.

Species	% abundance	% frequency/ 18 plots
*Penicillium thomii*Maire	10.8	100.0
Trichoderma polysporum (Link) Rifai	11.6	94.4
Trichoderma hamatum (Bon.) Bain.	5.8	94.4
Trichoderma viride Persoon: Fries	7.4	94.4
Geomyces pannorum (Link) Sigler & Carmichael	5.5	72.2
Penicillium spinulosum Thom	7.4	72.2
Penicillium sp. #1	4.2	72.2
Mortierella ramanniana (Mollller) Linnem v. *ramanniana*	6.5	66.7
Penicillium melinii (Thom)	4.4	61.1
Cylindrocarpon didymum (Hartig) Wollenweber)	1.9	55.6
Penicillium janthinellum Biourge	6.4	50.0
Mortierella sp. #5	1.1	50.0
Mucor hiemalis Wehmer	2.2	50.0
Chrysosporium merdarium (Link: Fries) Carmichael	1.4	44.4
Trichoderma koningii Oudem.	1.4	44.4
Penicillium janczewski Zaleski	2.7	44.4
Verticillium sp. #6	1.3	44.4
Nectria sp. #1	1.2	38.9
Paecilomyces carneus (Duché & Heim) Brown & Smith	1.2	38.9
Cylindrocarpon obtusisporum (Cooke & Harkness) Wallenw.	1.3	33.3
Penicillium brevicompactum Dierckx	0.9	33.3
Verticillium sp. # 2	0.6	27.8
Paecilomyces farinosus (Holm: Fries) Brown & Smith	0.9	27.8
Penicillium sp. #3	0.7	16.7
Verticillium sp. #10	0.4	5.6

Table 3. Morphological identifications, most similar Genbank accessions and percent sequence similarities for the microfungal species used in the multivariate analyses.

Morphological Identification [Accession #]	Sequence length (bp◊)	Genbank Match [Accession #]	Sequence similarity between our sequences & closest Genbank accessions (% identity)
Chrysosporium merdarium [DQ888721]	519	Uncultured ascomycete isolate [AY969783]	485/485 (100%)
Cylindrocarpon didymum [DQ888722]	515	*Cylindrocarpon didymum* [AY618228]	456/460 (99%)
Cylindrocarpon obtusisporum [DQ888723]	*518*	*Cylindrocarpon obtusisporum* [AY677292]	439/444 (98%)
Geomyces pannorum [DQ888720]	516	*Geomyces pannorum* [DQ189229]	499/501 (99%)
Mortierella ramanniana [DQ888724]	*603*	*Umbelopsis** sp. [AY376408]	575/590 (97%)
Mortierella sp. #5 [DQ888725]	614	*Mortierella* humilis [AJ878778]	586/590 (99%)
Mucor hiemalis [DQ888726]	620	*Mucor hiemalis f. silvaticus* [AY243948]	596/597 (99%)
Nectria sp. #1 [DQ888727]	589	*Nectriaceae sp.* [DQ317333]	471/476 (98%)
Paecilomyces carneus [DQ888728]	576	*Paecilomyces carneus* [AB103379]	567/569 (99%)
Paecilomyces farinosus [DQ888729]	593	*Paecilomyces farinosus* [AB083033]	573/577 (99%)
Penicillium sp. #1 [DQ888730]	490	*Penicillium kojigenum* [AF033489]	451/452 (99%)
Penicillium brevicompactum [DQ888731]	547	*Penicillium brevicompactum* [AY373898]	511/512 (99%)
Penicillium janczewski [DQ888732]	538	*Penicillium* sp. [AF125940]	513/534 (96%)

Table 3. Continued

Morphological Identification [Accession #]	Sequence length (bp◊)	Genbank Match [Accession #]	Sequence similarity between our sequences & closest Genbank accessions (% identity)
Penicillium janthinellum [DQ888733]	591	*Penicillium* sp. [AF178525]	538/545 (98%)
Penicillium janthinellum [DQ888733]	591	*Penicillium* sp. [AF178525]	538/545 (98%)
Penicillium melinii [DQ888734]	533	*Penicillium melinii* [AY373923]	525/527 (99%)
Penicillium spinulosum [DQ888735]	539	*Penicillium spinulosum* [AY373933]	518/522 (99%)
Penicillium thomii [DQ888736]	524	*Penicillium thomii* [AY373934]	516/518 (99%)
Penicillium sp. #3 [DQ888737]	590	*Penicillium lividum* [AY373922]	474/492 (96%)
Trichoderma hamatum [DQ888738]	619	*Trichoderma oblongisporum* [DQ083020]	510/533 (95%)
Trichoderma konigii [DQ888739]	594	*Trichoderma koningii* [AF055219]	539/546 (98%)
Trichoderma polysporum [DQ888740]	602	*Trichoderma album** [AJ608991]	551/572 (96%)
Trichoderma viride [DQ888741]	611	*Trichoderma viride* [AF456922]	529/535 (98%)
Verticillium sp. # 2 [DQ888742]	633	*Verticillium suchlasporium var. catenatum* [AB113353]	585/588 (99%)
Verticillium sp.# 6 [DQ888743]	561	*Verticillium bulbillosum* [AJ292410]	505/509 (99%)
Verticillium sp. #10	180	*Verticillium cf. suchlasporium* [AJ292400]	123/128 (96%)

◊ base pairs
*synonymous with the name obtained by morphological identification

Discussion

The most abundant and frequently isolated microfungal species from the plots were *Penicillium thomii*, *P.spinulosum*, *P. janthinellum*, *Penicillium* sp. #1, *P. melinii*, *Trichoderma polysporum*, *T. viride*, *T. hamatum*, *Mortierella ramanniana*, *Geomyces pannorum*, *Cylindrocarpon didymum*, *Mortierella* sp. #5 and *Mucor hiemalis*. Other studies of microfungal communities have also found these species to be common in forest soils. In a review of several forest microfungal community studies, Christensen (1981) states that *Mortierella* spp. and *Penicillium* spp. are characteristic of forest soils. In a study of the microfungal communities in forest soils in southern Québec (Widden 1986), where sampling took place in sites dominated by *Acer saccharum* (Marsh.), *Pinus strobus* (L.), and *Picea mariana* (Mill.) BSP, some of the main *Penicillium* species isolated included *P. thomii*, *P. spinulosum*, and *P. janthinellum*. *Trichoderma polysporum*, *T. viride*, *T. hamatum* and *Geomyces pannorum* were also common. A study of several coniferous soils in Canada (Widden and Parkinson 1973), lists *P. janthinellum and P.thomii*, among the most frequent *Penicillium* species isolated. Söderstrom and Bååth (1978) investigated the soil microfungal communities in Swedish coniferous forests and also frequently isolated *P. spinulosum*, *T. viride*, *T. polysporum*, *P. thomii*, *Mortierella ramanniana* and *G. pannorum*. *P.melinii* is commonly associated with acid soils (Widden 1987), and the average pH of the soil at our study site is 4.9.

Cylindrocarpon didymum may have preferences for colder regions as it is commonly isolated from arctic (Widden and Parkinson 1979) and alpine (Bisset

and Parkinson 1979) soils and was also common in this study. A study of the microfungi in Aspen forests in Saskatchewan lists *Mortierella* as the most common fungal genus (Morall 1974). Although *M. isabellina* and *M. vinacea* were listed as the most abundant, *M. ramanniana* was reported as frequent under aspen as well as spruce and birch in central Finland (Mclean and Huhta 2002).

The extraction of DNA from bulk soil and the use of molecular methods to analyze soil fungal communities are proving to be a valuable method for detecting novel taxa (Viaud et al 2000, Hunt et al 2004, Jumponnen and Johnson 2006). However, recent DNA based studies of soil microfungi reveal trends similar to those reported using culture based methods, with most of the sequences isolated belonging to the Ascomycota and most taxa being rare with only a few being very common (Jumponnen and Johnson 2006). As our main objective was to correlate the saprophytic microfungal community with the surrounding vegetation, the soil washing technique was selected as it was designed to isolate microfungal hyphae present in the soil. Amplification, cloning and sequencing of DNAs isolated from bulk soil would detect fungi from a wider range of taxa, including those from the Basidiomycota and Glomeromycota, but will not distinguish between fungal hyphae and the spore bank. In our previous study on the EM community (De Bellis et al 2006), a molecular based method was used to identify the fungi and although 207 taxa were detected, many (~76%) were rare and were removed from the analyses. When using canonical correlation analyses to observe relationships between the fungal community and

environmental variables, rare taxa are often not included in the analysis, in order to reduce the effect of these rare taxa.

Although we observed correlations between tree cover and EM fungi (De Bellis et al 2006) at the Lac Duparquet sites, none were seen in the case of the saprophytic microfungi. The mycorrhizal fungi, being symbiotic, obtain their carbon directly from their plant hosts and therefore their distributions are likely to be more tightly associated with their hosts. The mycorrhizal fungi may therefore be better buffered against the surrounding environmental fluctuations (Gehring et al 1998). Nantel and Newman (1992) found a high correlation between EM fungal sporocarps and host species in a mixed forest regardless of other soil characteristics. Villeneuve et al (1989) examined both the EM and saprotrophic macrofungi in forests along a south-north gradient in Québec where sampling took place in more floristically diverse deciduous sites in the south and less diverse coniferous forests in the north. The species richness of EM fungi remained relatively constant while the richness of the saprotrophic fungi declined. Also, the diversity of saprotrophic macrofungi was significantly related to the number of vascular plants, and the diversity of EM macrofungi was related to the percent cover of EM host trees.

The lack of observed correlations between distributions of microfungal and overstory tree species may be due to the fact that the plots of the 3 canopy types (birch, conifer, and aspen) were not pure enough to observe characteristic microfungal communities. In mixed vegetation sites, different plant species may selectively stimulate some fungal species in the surrounding soil (Westover et al

1997, El-Morsy 1999). The 18 plots in the present study had to have at least a 75% cover of birch, aspen or spruce and fir in the upper canopy to be considered a suitable plot (Fig. 2a). But, as the plots were set up in 1994, a lower canopy was present in most plots, therefore increasing the diversity of the vegetation present in each plot. The 1870 birch plots have a fairly high amount of mountain maple (*Acer spicatum* Lam.) in the lower canopy and the 1916 birch plots support a significant amount of fir in the overstory and lower canopies (Fig. 2b). The conifer plots were a mixture of spruce and fir, with the 1870 plots supporting mountain maple in the lower canopy and the 1916 plots supporting ~20% birch cover in the upper overstory canopy (Fig. 2b). In the RDA with the overstory and lower canopy used as the environmental matrix, although not significant, % overstory aspen was the variable that explained the most variation in the fungal species data. This could be due to the fact that the aspen plots were the least mixed plot types and had on average a 91% cover of aspen in the upper canopy.

The 18 soil cores can be grouped into 2 broad categories based on understory cover; those with high densities of *Aralia nudicaulis* and *Aster macrophyllus* L. as the main understory plants surrounding the core and those with lower densities of these herbs (Table 1). This division is seen in the RDA bi-plot with most plots with high *Aralia* and *Aster* clustering below Axis 1, and the other plots falling above Axis 1 and separating out relative to the other herbaceous plants. The plots that fall below Axis 1 are further separated, with the two plots with high amounts of *Aralia* falling away from the others. Previous studies on microbial communities have also reported changes in community structure associated with

small scale variations in plant cover. A study of the microbial communities in a mixed spruce-birch stand using phospholipid fatty acid (PLFA) profiles revealed that individual tree species affect the soil bacterial community, with patterns in the microbial community forming patches around spruce trees (Seatre and Bååth 2000). Westover et al (1997) also observed significant differences in the bacterial and fungal communities in the rhizosphere soil of different plant species.

A significant amount of variation in the microfungal community is explained by both the herbaceous plant community and soil chemistry. However, the variance partitioning analysis revealed that the microfungal communities were more closely correlated with the understory herb layer. There may be several reasons for the weak correlation between microfungal community and soil chemistry. The data available on the soil chemistry were collected for a previous study (Legaré et al 2001) and the measurements were taken from 4 representative cores from the entire $100m^2$ plot. Although the soil chemistry on this site is assumed to be relatively stable over a number of years (Brais et al 1995, Paré and Bergeron 1996), microfungi may be affected by small scale differences in soil chemistry. Chemical analysis of the same soil core from which the microfungi were isolated might have provided a more accurate picture of these relationships. However, the main objective of our study was to examine the relationships between the plant community and microfungal assemblages, and one of the main criteria for selecting the 18 plots was their location on similar clay deposits with similar soil chemistry. Differences in microfungal communities associated with soil chemistry

have been reported in cases where the soil chemistry varied greatly between the sites (Widden 1987). The understory plant community was significantly correlated with the microfungal species composition, explaining a significant part of the variability in the assemblages of microfungi found in these plots. However, other factors, not measured in this study, clearly also influence the structure of these communities.

Soils are made up of a mosaic of microhabitats and the organic soil layer is greatly dependant on the various types of litter inputs which in turn lead to variations in the quality of resources available to the saprophytic microfungi. Changes in resources can alter the structure of decomposer communities (Wardle and Lavelle 2002), and the relationship between plant inputs and available resources for soil microorganisms may explain the observed relationship between the microfungal community and understory plant species in these mixed-wood boreal plots.

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greatly thank Sarah McNair for her field assistance and Sonia Legaré for her soil chemistry data.

General conclusion

The main aim of this thesis was to examine the mycorrhizal and saprophytic microfungal communities in plots of different canopy types in the boreal mixed-wood forest. The data collected did in fact support the original hypotheses that the symbiotic ectomycorrhizae and microfungi would be correlated to the species composition of the aboveground plant community. Although both groups of fungi were correlated with the aboveground plant community, the ectomycorrhizae (ECM) were correlated with the various aboveground overstory plant hosts while the saprotrophic microfungi, varying at a much smaller spatial scale, were correlated to the species composition of the immediate aboveground understory herb layer. The arbuscular mycorrhizae are probably the group of fungi of the three studied in this thesis, of which we have the least amount of knowledge of their community structure in natural environments. Hence, in this study the aim was to obtain general knowledge about the community composition of arbuscular mycorrhizae in the boreal mixed-wood forest and to compare it with data collected from other natural environments.

Chapter 1 which examined the relationships between the ECM and the aboveground plant cover did indeed show that they were correlated with the aboveground canopy trees. As the ECM form a symbiotic association with the canopy trees it was originally predicted that a correlation between these should be detected. What sets this study apart from the many studies on ECM fungi is that it is one of the few studies that has examined the ECM community in an unmanaged forest environment, as most studies focus on the effects of a

disturbance, natural (i.e. forest fire, pest outbreak) or man-made (forestry practice, i.e. cut, liming) on the ECM community. This study also was one of the few that has analysed the ECM fungal community based on both morphological and molecular based methods and the data has revealed clear differences between the two. The results of this comparison showed that the morphological analysis may be biased to types that have resistant and easily identifiable mantles, whereas the molecular methods tend to detect a larger variety of taxa. Thus it could be concluded that both methods should be used to get a more accurate index of ECM fungal diversity in a particular ecosystem.

Due to the limitations in the isolation techniques available, direct comparisons of the arbuscular mycorrhizae with the plant community were not attempted. Therefore, for this group of fungi an alternative approach was used to analyse their community structure in the plots. *Clintonia borealis*, an herbaceous plant found in all plots that is colonized by arbuscular fungi was selected and molecular based methods were used to isolate AM fungi from the plant roots. The data collected in this study are particularity interesting as it is one of the first studies to examine the AM fungi in forests with canopies dominated by ECM tree hosts and it is first study that has looked at the AM fungi in a boreal forest in North America. No significant difference between the AMF communities from the *Clintonia* roots under the different canopy types were observed, and a particular sequence type was highly dominant in this system, making up approximately 70% of all the clones analyzed. Comparisons of this dominant sequence type with other published sequences from previous studies show that it clusters with

AM sequences collected from a variety of environments and hosts at distant geographical locations. Are certain types of AM fungi extremely common, being present in diverse environments in different plant hosts, or is this finding due to the fact that the primers used in the study target only a small group of AM fungi, or is it due to the fact that the 18S gene not variable enough at the species level? Although the primers may have some biases, it is known that these primers do pick up sequences from several AM genera and other studies using the same primer pair have not reported the most common AM type found in this study as the dominant type in their ecosystem. Another noteworthy finding from this study is that most of the AM types found do not match up with sequences obtained from morphologically identified AM fungi, indicating that communities of AM fungi are more diverse than initially thought based on spore morphology. Although the data collected in this chapter did shed some light on the study of AM fungi in boreal forests, it also further emphasizes our lack of knowledge of AM fungi in natural environments and should encourage future work on AM fungal ecology and genetics.

In Chapter 3 the saprophytic microfungi were examined from the same plots supporting the variety of overstory trees as sampled in Chapter 1. The soil microfungi are mainly litter-decomposers and it was predicted that their assemblages would differ at a much smaller scale and would therefore be correlated with the surrounding understory from where the soil sample was collected. This is likely one of the first studies to describe the relative importance of overstory tree composition and understory species composition on the

composition of the soil microfungal communities. Analyses of the data did indeed reveal that the microfungal assemblages were significantly correlated with the composition of understory plants in the plots. In this chapter, culture based methods were used to isolate the microfungi from the soil samples. Although this method was suitable to collect the data for the questions asked in this study, it is known that soils are likely to host a wide variety of fungi that could not be detected via the culture based methods. To further our understanding of the microfungal community, these data should be followed up with a study that would look at the general diversity and richness of the microfungi in these plots with both culture based and molecular based methods.

The data collected in this study showed how certain fungal groups are linked to the aboveground plant communities. The saprotrophic microfungi decompose dead plant material and release nutrients back into the soil for the aboveground plant community while the symbiotic mycorrhizae have a more direct effect as they colonize root tips and aid their plant hosts in nutrient and water uptake, pathogen resistance and more. Terrestrial ecosystems consist of above and belowground components that are linked and interact to influence ecosystem processes. Therefore, when studying forest ecosystems the belowground fungal community should not be neglected as they too play a major role in the maintenance of a healthy sustainable forest.

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Appendix 1. Genbank accession numbers corresponding to each sequenced RFLP type

Glomus group[a]	RFLP type No.	Genbank Accession No.
Group QU-Glo 1	1	DQ122650
	2	DQ122647
	6	DQ122653
	20	DQ122655
	30	DQ122651
	73	DQ122656
	74	DQ122674
	76	DQ122644
	82	DQ122648
	83	DQ122646
	84	DQ122645
	87	DQ122649
	94	DQ122652
Group QU-Glo 2	16	DQ122662
	17	DQ122664
	18	DQ122665
	19	DQ122669
	21	DQ122666
	22	DQ122661
	23	DQ122667
	43	DQ122668
	54	DQ122657
	70	DQ122659
	72	DQ122658
	75	DQ122660
	96	DQ122663
Group QU-Glo 3	10	DQ122622
	47	DQ122624
	49	DQ122623
	85	DQ122625
	88	DQ122626

Appendix 1, continued.

Glomus group[a]	RFLP type No.	Genbank Accession No.
Group QU-Glo 4	3	DQ122628
	7	DQ122631
	26	DQ122629
	51	DQ122627
	56	DQ122632
	77	DQ122630
Group QU-Glo 5	11	DQ122639
	55	DQ122640
	81	DQ122638
	93	DQ122641
Group QU-Glo 6	12	DQ122642
Group QU-Glo 7	14	DQ122637
Group QU-Glo 8	4	DQ122633
	5	DQ122634
	27	DQ122636
	35	DQ122635
Group QU-Glo 9	36	DQ122671
	50	DQ122673
	52	DQ122670
	91	DQ122672
Group QU-Glo 10	31	DQ122643

[a]Groupings correspond to those in Neighbour joining tree shown in Fig.2 of Chapter 2.