Microbial Ecology

Relationships between Stand Composition and Ectomycorrhizal Community Structure in Boreal Mixed-Wood Forests

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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 postfire ages and nine 100-m^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. A total of 1800 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 polymerase chain reaction 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 are influenced by 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.

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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 [60]. Ectomycorrhizal (ECM) fungi colonize the fine roots of a vast majority of the dominant tree species in the world's temperate and boreal forests [56]. A high diversity of ECM fungi may be necessary to maintain the stability and resilience of the forest ecosystem [54]. Both ECM fungal species diversity and composition have been shown to have important consequences for growth and nutrient uptake of the host plant [6, 37]. Thus, a highly diverse ECM community should more efficiently capture limiting resources and improve plant growth [38].

The factors influencing ECM fungal community structure and diversity are poorly understood. This may partly be explained by the fact that most studies on 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

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[26, 29], as well as biotic factors such as the proteolytic capabilities of the ECM fungi [16] and stand age [71], 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 [21, 65], and it is thought that different ECM fungi can colonize various locations along the root system, due to differences in available carbohydrates [13].

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) [46, 47]. Based on sporocarp production, Molina *et al.* [50] lists 11 ECM fungal species specific to *Betula*, 13 to *Abies* or *Picea*, and two to *Populus*. Nantel and Newman [51] 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.* [40] showed that the 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 attributable 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 [14, 38].

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 [11, 70], 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 [28].

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 [9], 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 [17, 23, 32], but boreal mixed-wood forests have only recently been explored in this respect.

Kernaghan *et al.* [40] 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.

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–*B. papyrifera* Marsh.) bioclimatic domain [31], 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 [25]. By dendrochronological analysis, Bergeron [8] and Dansereau and Bergeron [18] determined that the stands used in the present study originated from fires that took place 82–135 years ago. In the early stages of succession, paper birch (*B. papyrifera* Marsh.), trembling aspen (*P. tremuloides* 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 [*A. balsamea* (L.) Mill.] and white cedar (*Thuja occidentalis* L.) [44].

Sampling Design. A total of 18 sample plots, each measuring 10 × 10 m, situated on similar clay deposits were selected from an existing design [44]. 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.* [44] 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, three plots were selected in each of three forest canopy types: (1) trembling aspen dominated, (2) white birch dominated, and (3) 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 30 m 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 the fire, except for the 1870 aspen plots, which are a second cohort of aspen [9]. In August 2002, we reanalyzed 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, or spruce–fir, as recorded by Légare *et al.* [44], but a lower canopy layer of ~1.5 to 2 m 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 (~10 cm 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 4°C. The roots from a subsample 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, USA). 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, 10 root tips were selected from each square. Root tips that appeared senescent were removed prior to selecting the 100 root tips for the analysis. As soon as 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 200–400× magnification for further separation on the basis of microscopic features such as mantle pattern, hyphal morphology, presence or absence of clamp connections, and cystidia [1, 2, 30, 35]. 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 was extracted using the Qiagen Dneasy® Plant Mini Kit (Qiagen Inc., Mississauga, ON, Canada). 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 [27] and NL6C [41]. 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 pmol of each primer, and 2.5 units of *Taq* DNA polymerase. The thermal parameters used were similar to those cited in Gardes and Bruns [27]. The resulting PCR products were cloned using the p-gemT easy cloning kit (Promega Inc., Madison, WI, USA) following the manufacturer's instructions. Thirty-two positive clones of each sample were selected and reamplified with the primer pair used in the initial PCR. Glycerol stocks for each positive clone were prepared and tubes were stored at −80°C. A total of 576 cloned products were then digested with *Hin*fI, *Nde*II, and *Taq*I according to the manufacturer's instructions (Promega Inc.). 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 three gels were run for each plot). Agarose gels were examined on a GeneGenius Bioimaging system (Synoptics, Cambridge, England, UK) 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 100-bp plus molecular marker (MBI-Fermentas Corp.).

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 [22] with the default settings. RFLP types that were considered similar by GERM were always visually checked by comparing the band patterns on the actual gel photos. Once the analysis of each plot was complete, a data set containing only unique RFLP types was reanalyzed 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 (100 μL/mL) agar plates and grown overnight at 37°C. Examples of each RFLP type were reamplified 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 ~400-bp 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 neighbor-joining algorithm using PAUP*4.0b10 [64] was used to

infer the placement of our sequences relative to the reference sequences obtained from Genbank. ClustalX 1.81 [67] 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 [33]. The tree was rooted with a *Glomus mosseae* sequence obtained from Genbank (Accession AY236334).

ECM RFLP Richness and Diversity. Ectomycor-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 [24] and morphotypes [57] may not always equate to individual species, they were treated as operational taxonomic units to calculate Shannon diversity [58] and richness indices.

Ordination Analysis. Canonical correspondence analysis (CCA) [66] 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) [36]. 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 [48].

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 nine plots, and the *Amphinema*-like were found in 10 of the 18 plots. From this analysis, we found three morphotypes unique to the conifer-dominated plots, two 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 *Taq*1 patterns. The most common RFLP type occurred 65 times (11.3%), and was present in five out of the 18 plots. Ten other RFLP types occurred more than 10 times, 23 RFLP types occurred between three and nine times, and 15 types occurred twice. 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, at $H' = 3.0$. 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 nonmycorrhizal 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 nonmycorrhizal species may be amplified. Forty-eight 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 nonmycorrhizal 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 neighbor-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 18 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

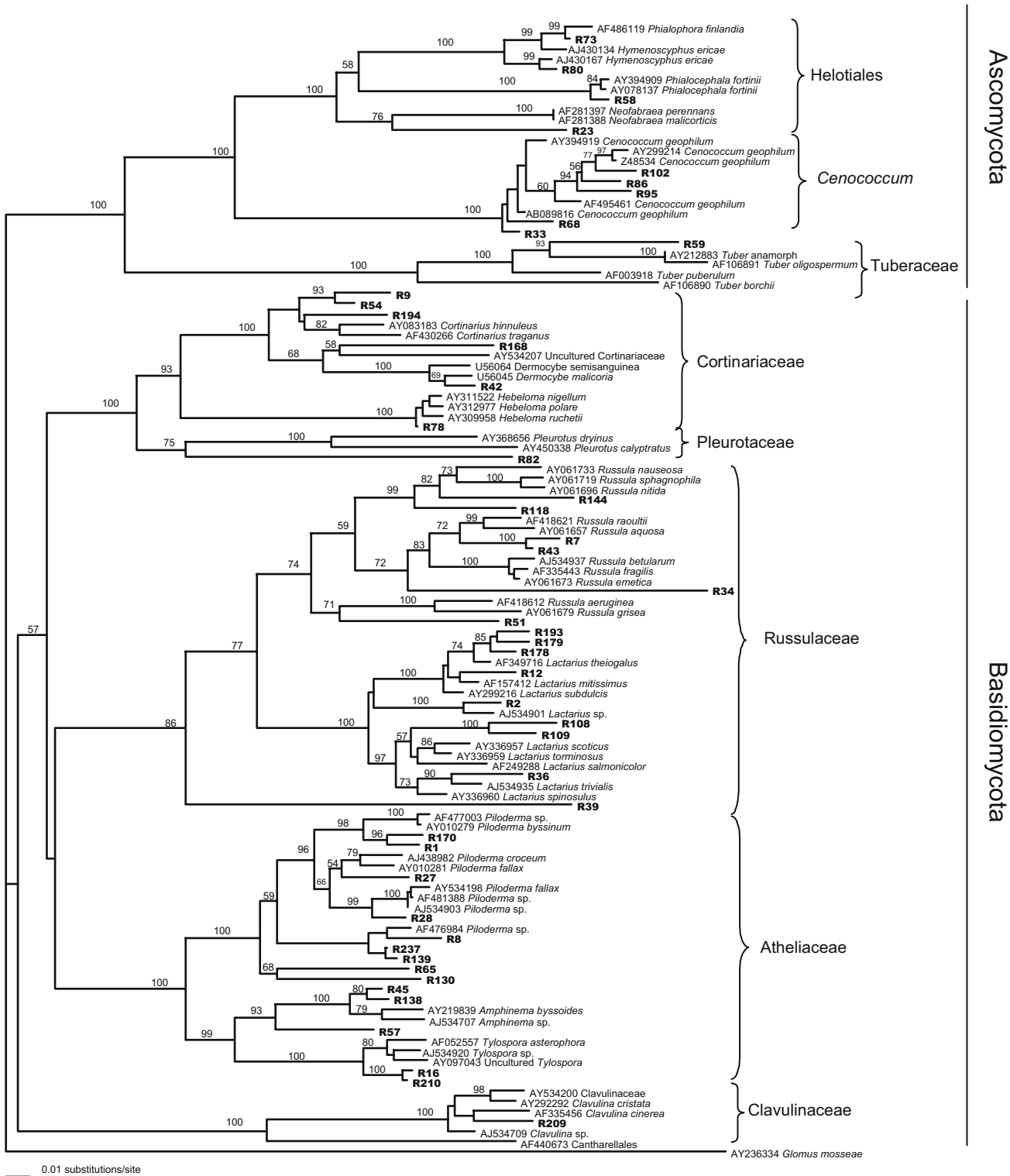

Figure 1. Neighbor-joining tree demonstrating the placement of the sequenced RFLP types with reference sequences obtained from Genbank. Sequences from Genbank are identified with their accession numbers. Sequenced RFLP types are shown in bold. Bootstrap values (1000 replicates) are shown at the nodes.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

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 intraset correlations are used to infer which environmental factors are contributing most to each axis [64]. Hence, we can infer that axis 1, which accounts for most of the variation, represents a birch–spruce gradient. The first three 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 (four environmental variables). The CCA was also performed using the entire RFLP dataset (207 RFLP types) and % overstory composition, with similar results (not shown).

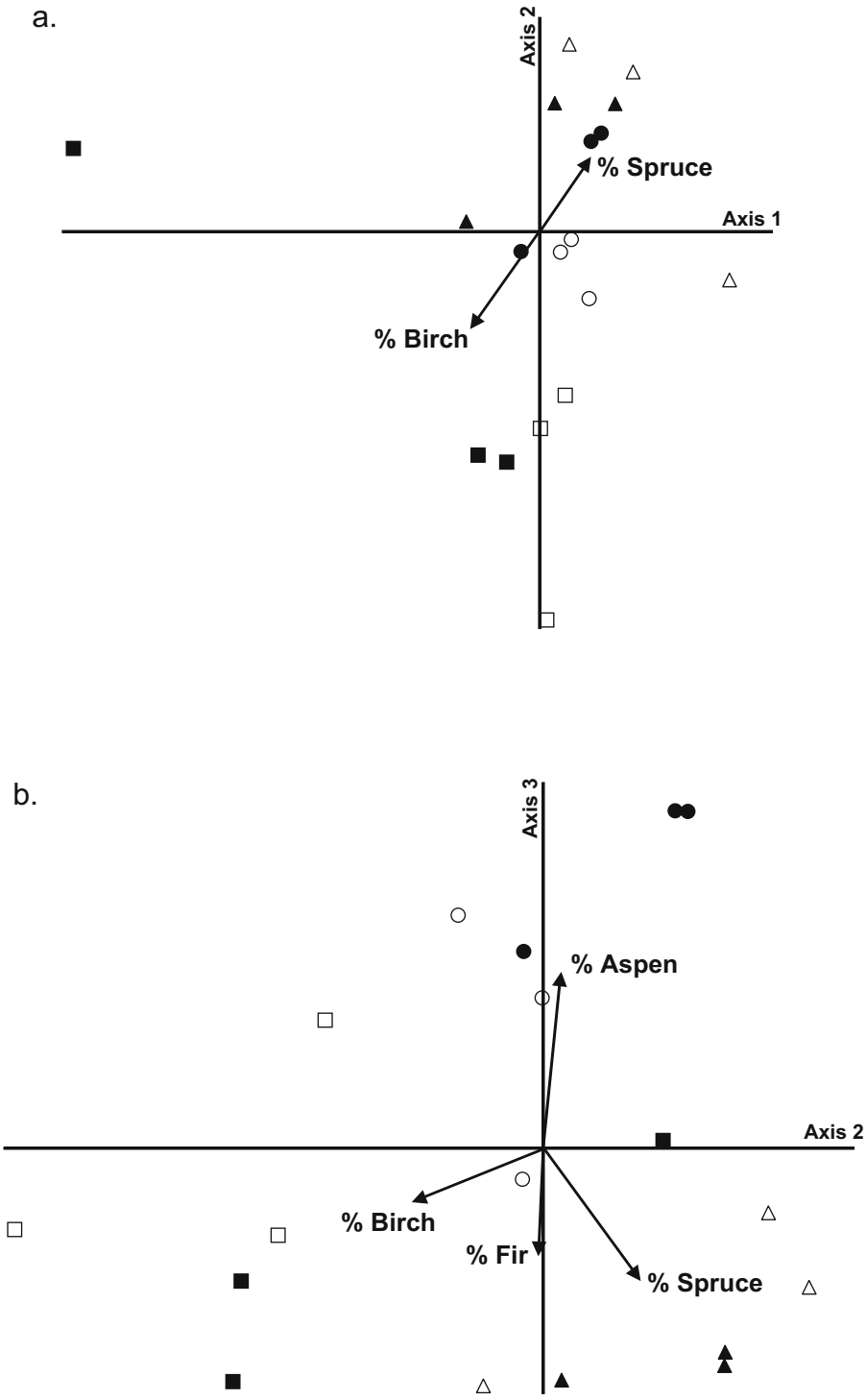

Figure 2. Canonical correspondence analysis of the ECM community (based on the molecular identification) from the 18 plots supporting the various stand types of two postfire ages. *Circles* represent the aspen plots, *triangles* represent the conifer plots, and the *squares* represent the birch plots.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

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 three 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 three axes of the CCA accounts for 49.4% of the total variance in the species data with respect to the overstory tree assemblages (intraset correlations for the 1916 plots

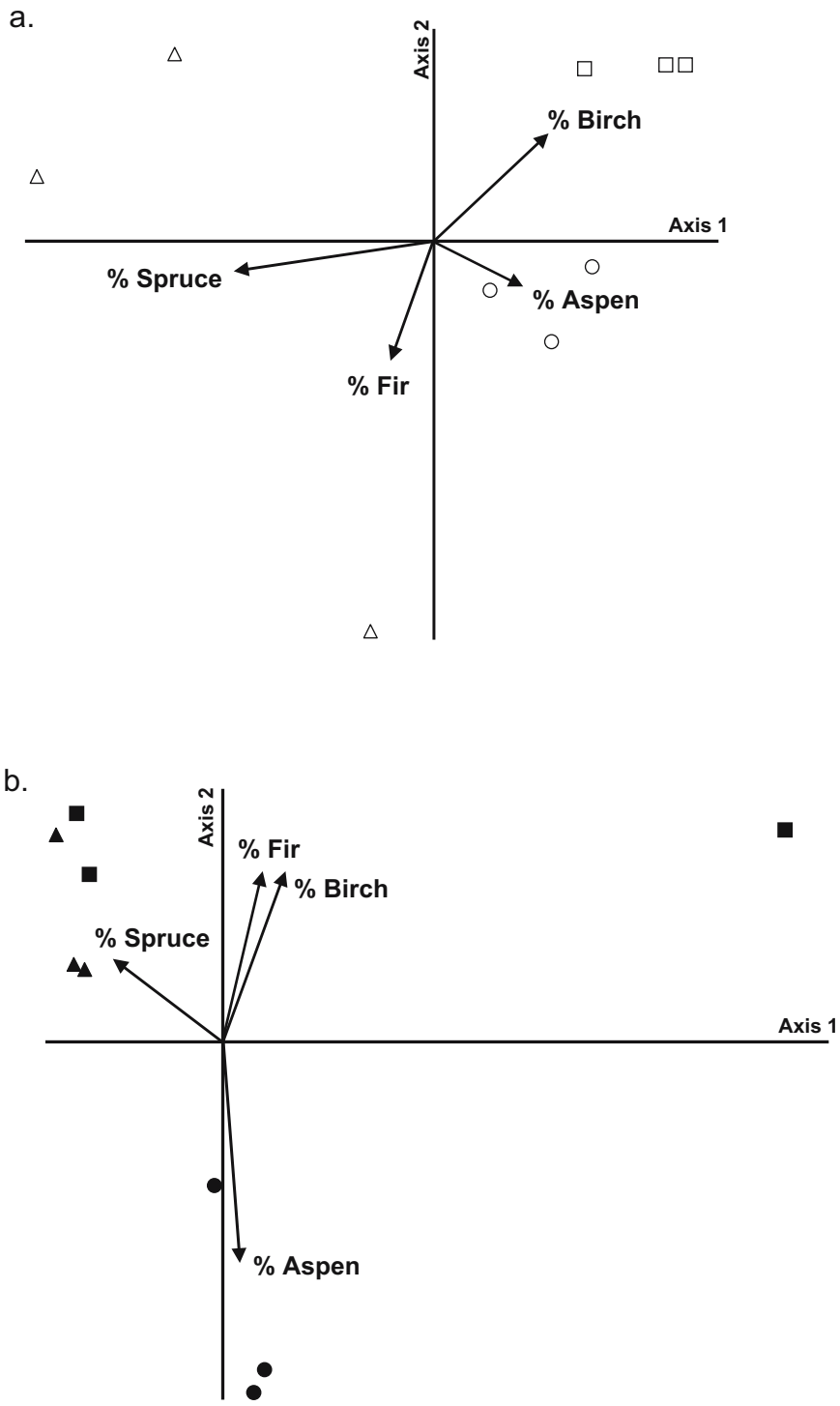

Figure 3. Canonical correspondence analysis of the ECM community (based on the molecular classification) of plots of similar age groups supporting the various stand types. *Circles* represent the aspen plots, *triangles* represent the conifer plots, and *squares* represent the birch plots. (a) Axes 1 and 2 of the biplot of the 1870 plots, (b) axes 1 and 2 of the biblot of 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: axis 1, $r = 0.990$; axis 2, $r = 0.979$). The eigenvalues for the first two axes were 0.892 and 0.809. The main difference between the two biplots is a better separation between the birch- and conifer-dominated plots in the biplot 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, whereas 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 postfire 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 ($\lambda_1 = 0.40$, $\lambda_2 = 0.31$; Fig. 5). Table 1 shows the number of plots in which each of the ECM RFLP types were found.

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 (Figs. 2, 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 three 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, whereas 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

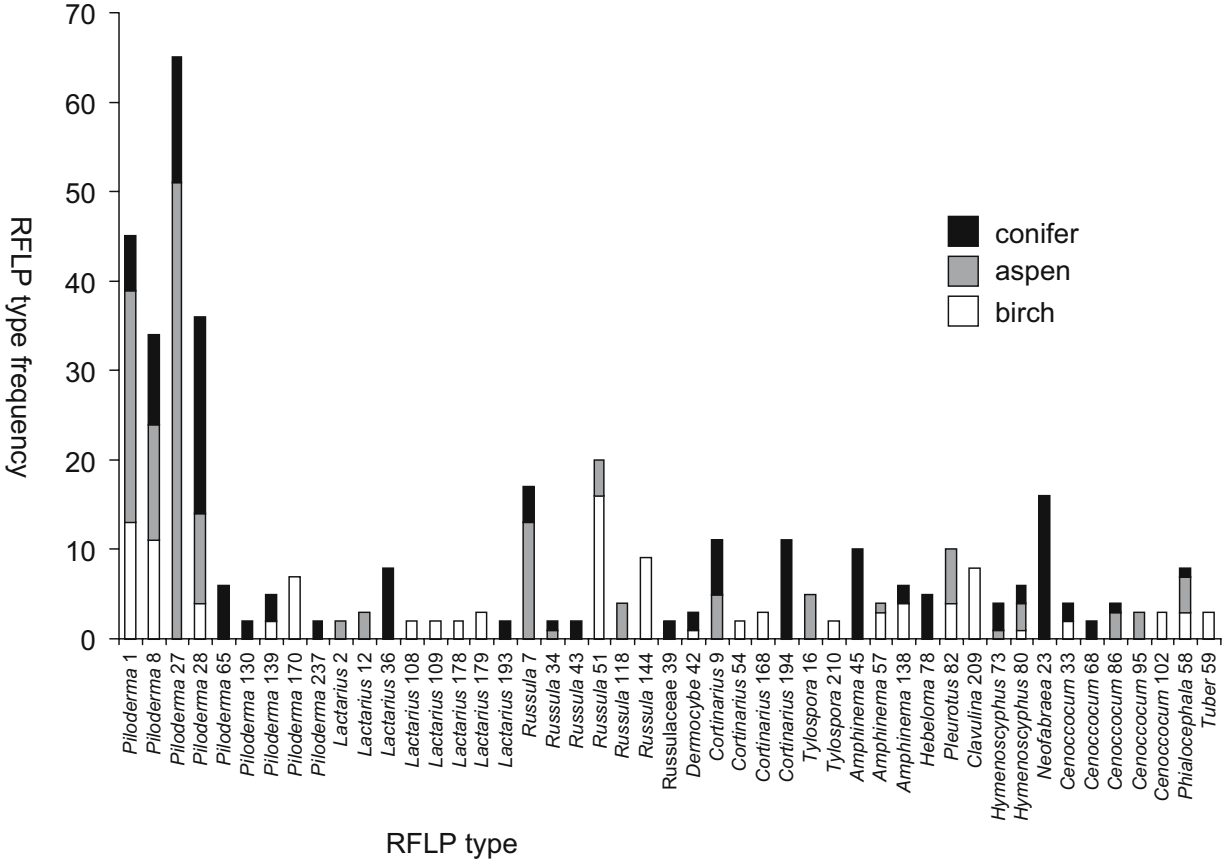

Figure 4. Frequency of each sequenced RFLP type per canopy type. Bars represent the number of times a certain RFLP type was found in the pooled dataset (from stands in both post fire ages) of plots of similar canopy type ($n = 6$). Each sequence is assigned to a genus on the basis of its closest match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

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

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 [46], brought about by differences in root and litter inputs [14, 38], or perhaps to differences in patterns of belowground resource allocation (see [5]). 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 preference for certain stand types (Figs. 4 and 5). Not only did the birch-dominated plots appear quite distinct, with most plots clustering together in the biplots (Fig. 2), they also supported the highest ECM fungal RFLP richness and diversity. Other studies have also reported distinct microbial communities in birch soils; Smolander [62] showed increased densities of *Frankia* sp., DeLong *et al.* [20] reported increases in pseudomonads in birch soils, and Pirha *et al.* [55] found more fungal specific fatty acids in birch rhizosphere soils vs those of pine and spruce. Suitable carbon sources are thought to be the most limiting factor to soil microbes [72]; hence the increased ECM fungal richness and diversity may be related to the easily leached water soluble compounds released from birch litter [7] and to the root exudates of birch roots, which have been reported to have high amounts of carbohydrates [59]. Studies of mixed conifer–birch stands report improved soil conditions [12] and enhanced productivity of the cooccurring conifers [45], which may be partly 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 [68]; however, 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 [68] lists several *Picea*-specific ECM fungal species (on the basis of fruiting bodies), and Molina and Trappe [49] demonstrated differences among ECM fungal species in their ability to colonize different conifer species *in vitro*. Koide *et al.* [42] and Baar and de Vries [4] suggest that the chemistry of forest litter can affect ECM fungal communities. Conifer litter contains high levels of phenolics [63], and decomposes relatively slowly [53], resulting in an accumulation of recalcitrant forest floor material [52], which may influence the ECM fungal community. Although fir is also known to support characteristic ECM fungal species [39, 49, 68], 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 (Fig. 3a, b). Although aspen is associated with some unique ECM fungal species [15, 50], their roots have been shown to support a lower ECM fungal diversity than any other host tree in our study area [40]. The aspen trees in our study area represent the second postfire cohort and are therefore younger than the other host trees. However, aspen can survive belowground after fire and quickly recolonize 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 *Amphenema* (RFLP #78) were most abundant in birch

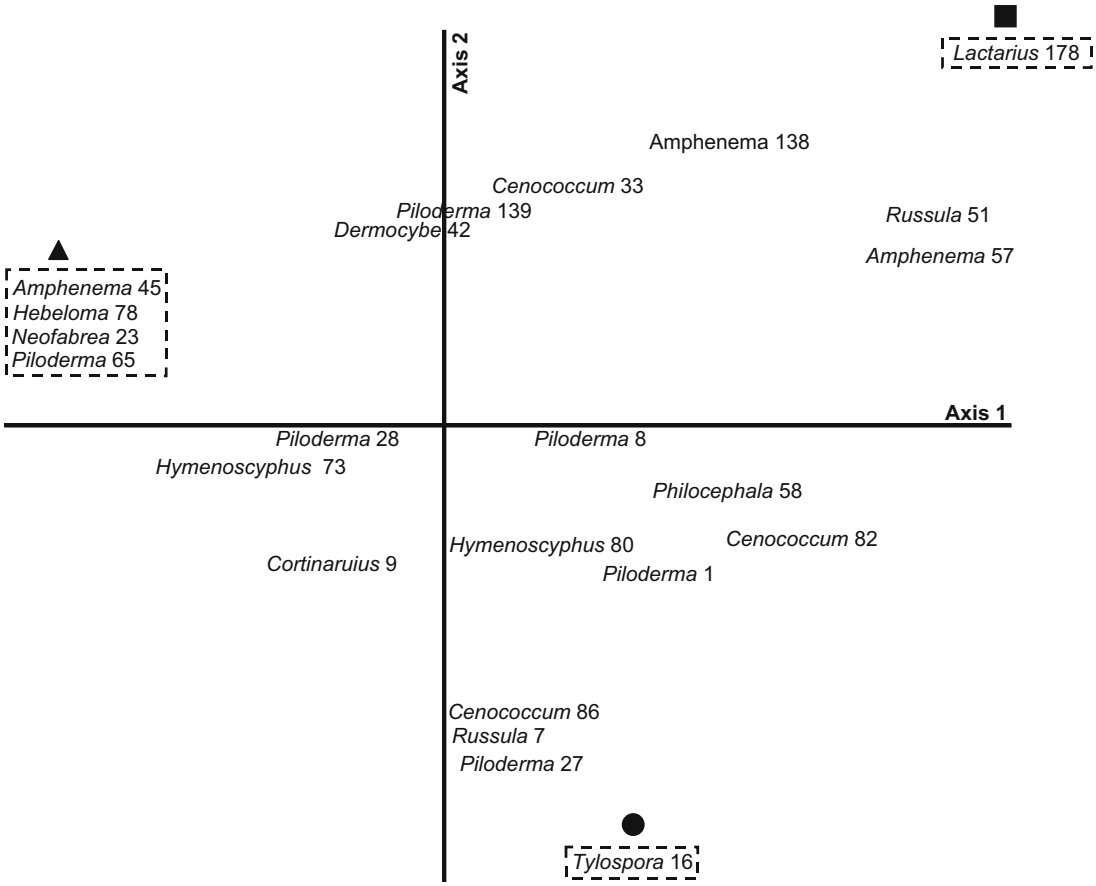

Figure 5. Correspondence analysis of the three canopy types and sequenced RFLP types that occurred in more than one plot. *Boxed data* represents types that were found exclusively in plots of a single canopy type. The *circle* represents aspen-dominated plots, the *triangle* represents conifer-dominated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

plots. *Piloderma* (RFLP #65), *Hebeloma* (RFLP #78), *Amphenema* (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 *C. geophilum* and the thelephoroid fungi. The morphological analysis in the present study, and that of Kernaghan *et al.* [40] (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 *C. geophilum*, and none of the sequenced clones matched thelephoroid fungi. Both *C. geophilum* and members of the Thelephoraceae form robust mantles and are easily identified morphologically [30, 43]. This may allow for their identification even after the fungal or host tissues are dead [69]. 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.* [41], 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 Sakakibara *et al.* [57], 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 [26, 34]. 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 on ECM fungal communities from boreal forests [17].

Piloderma spp. are often common in old growth coniferous forests [16, 31], and their occurrence has been correlated with coarse woody debris abundance [61]. 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 [16]. 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 [52]. *Russula* species are also late successional ECM fungi [19] that are known to have the potential to release peroxidases and/or polyphenol oxidase [3].

Our results indicate that overstory tree species composition is related to the belowground community of ECM fungi, possibly attributable 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 [10]. 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 [10]. 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.

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