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Plant community influences on soil microfungal assemblages in boreal mixed-wood forests

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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 18 100 m^2 plots from an existing research site. Plots were separated into three 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 1 m^2 subplot was established in which the understory herbaceous layer was surveyed and soil cores were collected. Microfungi were isolated from soil cores with the soil-washing technique and isolates were identified morphologically. To support our morphological identifications DNA sequences were 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.

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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 2002) 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). Studies also have 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. A microfungal community analysis of alpine soils by Bisset and Parkinson (1979) similarly indicated that the major source of variation in microfungal species composition is attributable to differences among sites, which largely are 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. Because soil chemical analyses already had been conducted (Legaré et al 2001) we selected plots on similar clay deposits that had similar chemical properties 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 had focused previously on the ectomycorrhizal (EM) fungal communities at this site and found strong positive correlations among overstory tree composition and diversity and EM fungal species composition and diversity (Kernaghan et al 2003, De Bellis 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.

Because EM fungi form a symbiotic relationship with their plant hosts, correlations between these fungi and canopy trees was expected. However, because 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 proximity. We therefore hypothesized that, unlike the EM fungi, the assemblages of microfungi would be correlated more strongly with the immediately surrounding understory vegetation than with canopy trees.

MATERIALS AND METHODS

Site description.—The study area is 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 bioclimatic domain (Grondin 1996), which extends over the clay belt region of Québec and Ontario. The closest weather station is 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 after fires 82–135 y 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 or from fires in 1916 or 1923. Half of the cores were collected from plots from the 1870 fire and half from the 1916/1923 fires, (hereafter referred to as the 1916 plots). Within both the 1870 and 1916/1923 sites, we selected 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. 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 three 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 initially was set up in 1994 (Legaré et al 2001). In Aug 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 still was dominated (>75%) by either trembling aspen, white birch or spruce-fir, as recorded by Legaré et al (2001), but we also noted a lower canopy layer of ~2 m 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 heterogeneity of the understory vegetation, a 1 m^2 subplot was arbitrary marked at the southwest corner within each 100 m^2 plot and the percent cover of each understory plant (<1 m tall, including tree seedlings) was estimated. Plants >1 m tall were considered as the lower canopy. In the field, when the 1 m^2 plot was established, all understory plants were identified and by visual observation percent cover was recorded. Also a digital photo of each 1 m^2 plot was taken. Later the percent 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.—Data was taken from a previous study in which four samples were taken from the FH horizon, pooled within plots, air-dried, then ground before analyses for pH, total Ca, K, Mn, Mg, P, N and organic carbon (Legaré et al 2001).

Sampling, isolation and identification of fungi.—In Aug 2004 a core of organic soil (~10 cm deep) was taken with a 7.5 cm diam corer from each of the 18 1 m^2 subplots described above. 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 soil microfungi were enumerated with the soil-washing method (Parkinson and Williams 1961). Five gram subsamples from each soil core were washed with 20 1 min cycles 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 d and each fungal colony was identified morphologically and counted. Colonies that were not readily identified were subcultured onto 2% malt-extract

agar (Oxoid Ltd., Code LP0039) for future identification. Sporulating soil microfungi were identified with keys including those of Domsch and Gams (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 microfungi.—DNA was extracted from the 25 most common microfungi. Isolates were grown on 2% malt agar for 2 wk. Mycelia then were removed and the DNA extracted with a modification of the protocol outlined by Gardes and Bruns (1993). Tissue was ground in liquid nitrogen in a ceramic mortar and incubated 1 h at 65 C in 600 µL 2 × 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 then was 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 resuspended in 50 µL water. The ITS1-5.8S-1TS2 region of the ribosomal DNA was amplified with 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, 2.5 mM MgCl 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 McGill University and Genome Québec Innovation Centre with 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 among overstory tree composition, understory vegetation, soil chemistry and the microfungal species composition were assessed with 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 datasets. More specifically in this case a matrix of explanatory variables (percent 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 stepwise 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 with the computer package CANOCO 4.5 (ter Braak and Smilauer 2002).

Variance partitioning.—RDA with the microfungal species data and all three 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 four parts, (i) variation solely due to soil chemistry, (ii) variation solely due to understory plants, (iii) shared variation of the two environmental matrices and (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 with the Monte Carlo permutation test with 999 permutations. All analyses were carried out with 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 1 m^2 plots. To investigate relationships between the microfungal community and the understory community, plants present in <5% of the plots were 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* and *Aralia nudicaulis* L. were the understory plants that accounted for most of the variation in the microfungal species data. The understory species richness in each plot, as well as the percent cover of each plant used in the RDA including *Aster macrophyllus* L. because it was abundant in several plots is provided (TABLE I).

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 respectively 44, 43 and 48 for the birch, conifer and aspen dominated plots. *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 II). Along with the microfungi mentioned above, *Trichoderma hamatum, Geomyces pannorum, Penicillium* sp. No. 1, *Penicillium melinii, Cylindrocarpon didymum, Mortierella* sp. No. 5 and *Mucor hiemalis* also were fairly common, being present in more than 50% of the plots (TABLE II).

TABLE I. Understory richness (R) and percent cover of the main understory plants present in the eighteen 1 m² 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

Microfungal isolates were identified morphologically and the most common isolates were characterized molecularly. DNA sequences were obtained for the 25 most common microfungi to confirm our morphological identifications. Sequences were approximately 550 bp long, with the exception of the sequence obtained for *Verticillium* sp. No. 10, which was only 180 bp. All sequences except for the one obtained for *Verticillium* sp. No. 10 have been submitted to GenBank and all were compared to those in GenBank using BLAST to obtain closely matching sequences (TABLE III). Of these 25 microfungi, 18 were identified to species and seven were identified to genus based on the morphological analysis (TABLE III). 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 two *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 III). We also performed reverse searches for these sequences. We searched GenBank for sequences identified as these three species and the sequences were compared. *P. janczewski* had a 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 two introns in the ITS 1 region that were not present in the GenBank sequences and this resulted in a lowered sequence identify for the whole length of the sequence. Four isolates not identified to species using morphological methods, *Mortierella* sp. No. 5, *Penicillium* sp. No. 1 and *Verticillium* spp. Nos. 2 and 6, had a 99% sequence identity match with *Mortierella humilis, Penicillium kojigenum, Verticillium suchlasporium* and *Verticillium bulbillosum* respectively, and *Penicillium* sp. No. 3 had 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 because 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 high sequence similarity to other GenBank sequences identified as either *Geomyces pannorum* or *Pseudogymnoascus roseus*. We do not think our identified *C.*

TABLE II. 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 : Fr.) Rifai	11.6	94.4
Trichoderma hamatum (Bon.) Bain.	5.8	94.4
Trichoderma viride Persoon	7.4	94.4
Geomyces pannorum (Link) Sigler & Carmichael	5.5	72.2
*Penicillium spinulosu*m 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) Wollenw.	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 ex S. F Gray) Brown & Smith	0.9	27.8
Penicillium sp. #3	0.7	16.7
Verticillium sp. #10	0.4	5.6

merdarium is *G. pannorum* because we also isolated *G. pannorum* and it clearly was distinguishable from *C. merdarium* based on morphological features. Although we obtained a short sequence for *Verticillium* sp. No. 10, its closest match from GenBank was a *Verticillium* species. Our isolates of *Trichoderma hamatum* (based on morphological identification) had a 95% sequence similarity to *T. oblongisporum* (TABLE III). These two species have similar morphological traits and would have been regarded by Rifai (1969) as members of the *T. hamatum* species aggregate, which later was 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 five 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 stepwise forward selection showed that none of the variables were significantly related to the fungal community data, however the variable that contributed the most to the species variation was percent overstory aspen cover (P = 0.12). The resulting RDA with percent 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 with 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 and 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 that accounted for most of the variation in the microfungal species

TABLE III. 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%)
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.

data. RDA with these four species produced an ordination in which the first and all canonical axes were significant ($P = 0.001$ and $P = 0.001$, 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% by the second. The intraset correlations between the environmental variables were examined to determine those 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 percent *Rubus* and percent *Corylus*. In the RDA biplot (FIG. 1) we see a separation along the first axis with the two

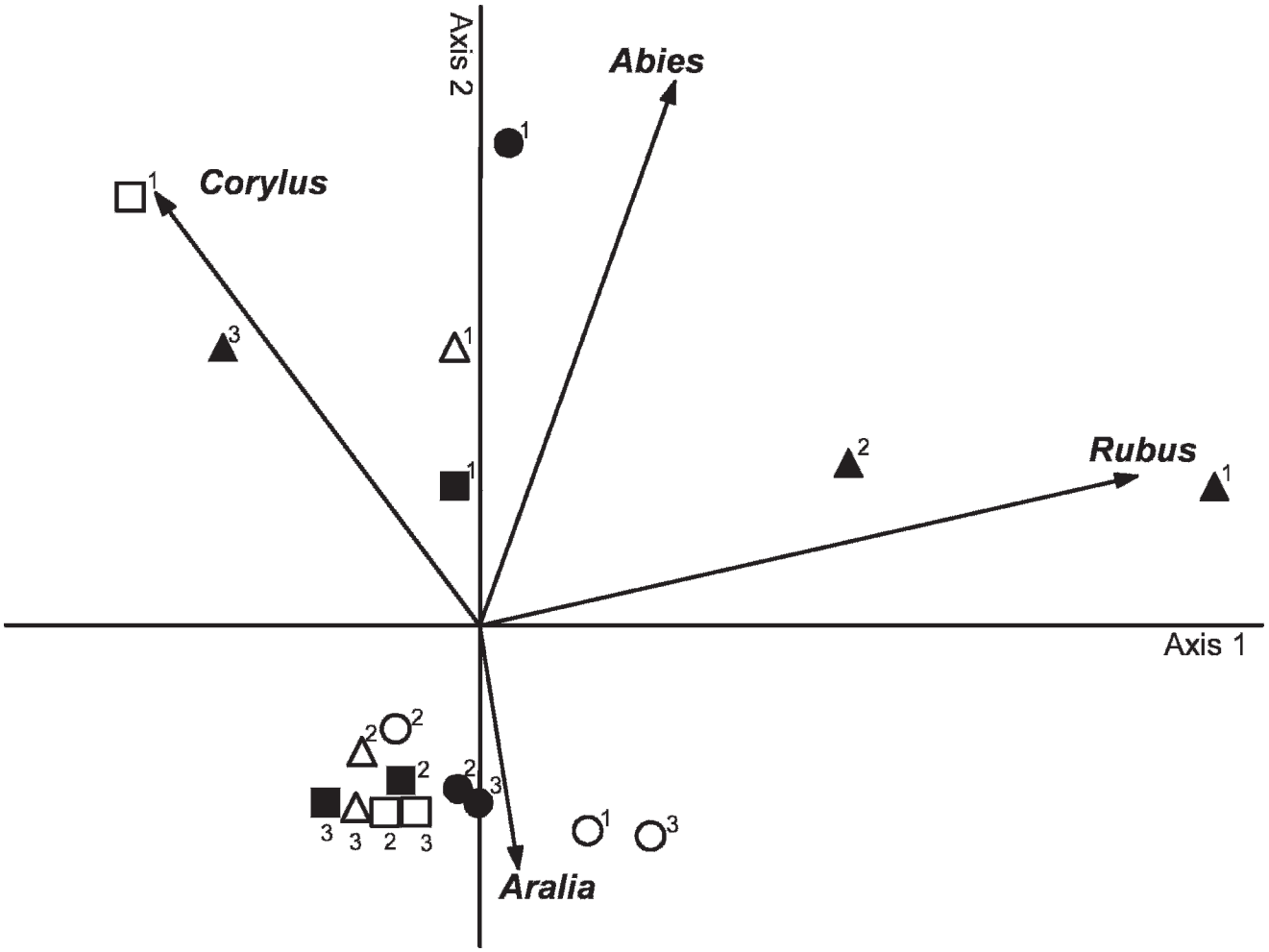

FIG. 1. Redundancy analysis of the microfungal community (based on the 25 most frequent species) and understory vegetation from the 18 plots. Circles represent the aspen plots, triangles represent conifer plots and squares represent birch plots. Open symbols represent plots from the 1870 fire, while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

plots with the highest amount of *Corylus* (intraset correlation −0.42) toward the negative direction of the first axis and the two plots with the most *Rubus* falling toward the positive (intraset correlation 0.85). The second axis was most correlated with percent *Abies* and percent *Aralia*. Along the second axis plots with *Abies* and no *Aralia* (1870 conifer replicate No. 1 and 1916 birch replicate No. 1, FIG. 1) are in the positive direction of axis 2, with *Abies* having an intraset correlation of 0.71 with axis 2, while the 1916 aspen plot replicate No. 1 is closer to the center because 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 intraset correlation of −0.31. The plots with the highest amount of *Aralia* fall toward the negative direction of axis 2 (FIG. 1). Most of the other plots in the negative direction of axis 2 consisted of plots dominated by *Aster macrophyllus* 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, accounting for 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.

DISCUSSION

The most abundant and frequently isolated microfungal species from the plots were *Penicillium thomii, P.spinulosum, P. janthinellum, Penicillium* sp. No. 1, *P. melinii, Trichoderma polysporum, T. viride, T. hamatum, Mortierella ramanniana, Geomyces pannorum, Cylindrocarpon didymum, Mortierella* sp. No. 5 and *Mucor hiemalis*. Other studies of microfungal communities have 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* (P. 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 might have preferences for colder regions because it is isolated commonly from arctic (Widden and Parkinson 1979) and alpine (Bisset and Parkinson 1979) soils and was 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 is 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. Thus Jumponnen and Johnson (2006) showed that most of the sequences isolated belonged to the Ascomycota and that most taxa were rare with only a few being very common. Because our main objective was to correlate the saprophytic microfungal community with the surrounding vegetation, the soil-washing technique was selected because it was designed to isolate microfungal hyphae. 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 often are not included in the analysis 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 those of their hosts. The mycorrhizal fungi therefore might be better buffered against 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, but 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 might be due to the fact that the plots of the three canopy types (birch, conifer and aspen) were not pure enough to observe characteristic microfungal communities. In mixed vegetation sites, different plant species might selectively stimulate some fungal species in the surrounding soil (Westover et al 1997, El-Morsy et al 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 because the plots were

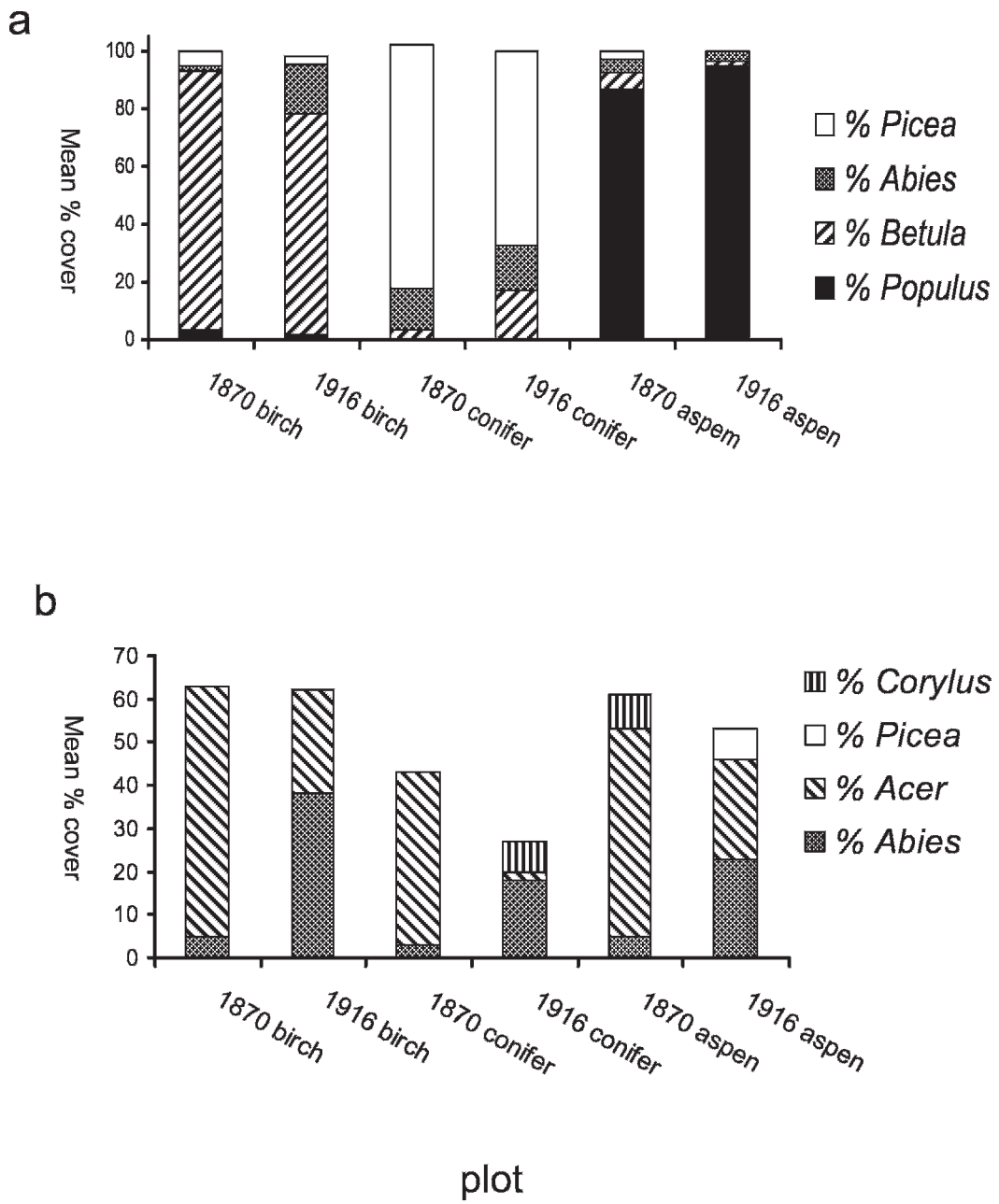

FIG. 2. Analysis of the (a) overstory and (b) lower canopy for each plot type. The percent cover for each species is an average value of the three 100 m^2 plots from the 1870 fire and the 1916 fire sites. Legend shows the genus names for each of these species: *Picea glauca, Abies balsamea, Betula papyrifera,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

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, the percentage of 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 two broad categories based on understory cover, those with high

densities of *Aralia nudicaulis* and *Aster macrophyllus* as the main understory plants surrounding the core and those with lower densities of these herbs (TABLE I). This division is seen in the RDA biplot 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 separated further, with the two plots with high amounts of *Aralia* falling away from the others. Previous studies on microbial communities also have 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 study by Legaré et al (2001) and the measurements were taken from four representative cores from the entire 100 m^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 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 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 might explain the observed relationship between the microfungal community and understory plant species in these mixed wood boreal plots.

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