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Diversity and species distribution of ectomycorrhizal fungi along productivity gradients of a southern boreal forest

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Abstract Coniferous forests with diverse ectomycorrhizal fungus (EMF) communities are associated with nutrient-poor, acidic soils but there is some debate whether EMF can be equally adapted to more productive, nitrogen-rich sites. We compared EMF species distribution and diversity along a replicated productivity gradient in a southern boreal forest of British Columbia (Canada). Roots from subalpine fir (*Abies lasiocarpa*) saplings of the understory were sampled and EMF species were identified by morphotypes supplemented with ITS rDNA analysis. There were significant changes in the distribution and abundance of 74 EMF species along the productivity gradient, with as little as 24% community similarity among contrasting sites. Species richness per plot increased asymptotically with foliar nitrogen concentrations of subalpine fir, demonstrating that many EMF species were well suited to soils with high rates of nitrogen mineralization. EMF species abundance in relation to site productivity included parabolic, negative linear, and positive exponential curves. Both multi-site and more narrowly distributed EMF were documented, and a diverse mix of mantle exploration types was present across the entire productivity gradient. The results demonstrate strong associations of EMF fungal species with edaphic characteristics, especially nitrogen availability, and a specialization in EMF communities that may contribute to the successful exploitation of such contrasting extremes in soil fertility by a single tree host.

Keywords Ectomycorrhizal species richness · Nitrogen · Boreal forests · Diversity–productivity relationships · Ectomycorrhizal exploration type

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Introduction

Ectomycorrhizal fungi (EMF) are the key mediating agent between soils and many tree species, and research into the diverse communities EMF may form continues to expand upon abiotic–biotic relationships fundamental to forest ecology. These investigations include the association of particular EMF assemblages with edaphic and climatic factors (Gehring et al. 2006), the role of EMF species and fungal networks in forest nutrition and productivity (Paul et al. 2007; Selosse et al. 2006), and the dynamics of EMF communities in primary or secondary forest succession (Nara 2006; Twieg et al. 2007). Ultimately, the insights into EMF community ecology gained from these lines of inquiry should provide a better understanding of forest soil ecosystems and tree species autecology (especially survival, nutrition, and productivity), and enable a more thorough evaluation of forest ecosystem response to stressors such as forest harvesting, atmospheric pollution, invasive species, and climate change.

One fundamental aspect of EMF ecology is the relationship between soil nitrogen (N) supply and EMF species distribution and diversity. It is increasingly apparent that plant nutrition in cold, less productive forests is dependent on organic N to a large degree (Lipson and Näsholm 2001), and that many EMF of boreal and subalpine forests can facilitate organic N availability and uptake (Chalot and Brun 1998; Read and Perez-Moreno 2003). In addition, a number of experimental studies with N fertilizer or of atmospheric N deposition have demonstrated large shifts in EMF species distribution with increased inorganic N availability (Peter et al. 2001; Lilleskov et al. 2002; Avis et al. 2003) and often losses in 'specialist' or stress-tolerant EMF species (Wallenda and Kottke 1998; Taylor et al. 2000). These results suggest, at least for conifer species, that a primary niche of EMF is nutrient-poor, acidic organic soils with negligible rates of N mineralization (Read et al. 2004). For these reasons, we might expect EMF diversity in coniferous forests to decline with increasing soil N availability (Parrent et al. 2006; Taniguchi et al. 2007), to the extent even of non-mycorrhizal root proliferation (Berch et al. 2006), and shifts in forest dynamics to favor arbuscular mycorrhizal plant and tree species (Nilsson et al. 2005).

Alternatively, many conifer species establish across quite wide gradients in soil moisture or nutrient regimes, and investigations of more pristine habitat have revealed an array of EMF species able to thrive on N-rich sites (Toljander et al. 2006). Rather than changes in simply species richness, the effect of soil fertility might be revealed through shifts in the distribution of genera such as *Cortinarius* and *Tricholoma* (Trudell and Edmonds 2004), in the functional attributes suggested by mantle characteristics (Nilsson and Wallander 2003), or in the abundance of mushroom fruiting (Kårén and Nylund 1997; Jonsson et al. 2000). Few studies have thoroughly examined EMF communities across naturally contrasting soils or habitat types, but it is apparent that both widely tolerant, generalist species and more niche-specialized species can be expected within mature forest landscapes (Nantel and Neumann 1992; Gehring et al. 1998; Kernaghan and Harper 2001; Toljander et al. 2006; Robertson et al. 2006). Soil N availability can vary temporally during cycles of forest disturbance as well, although the duration of this effect and influence on EMF communities appears to be subtle (Kranabetter et al. 2005; Yamashita et al. 2007; B. Twieg, unpublished).

Detailed study of EMF species distribution across well-defined and replicated natural edaphic gradients would help clarify the significance of soil fertility to EMF communities. One such gradient was described for upland plant associations of southern boreal forests in British Columbia (Canada), where stand productivity and foliar N concentrations were positively correlated to dissolved organic N mass and N mineralization rates of the soil profile (Kranabetter et al. 2007). In addition, key differences in soil biota were suggested by forest floor morphology (Green et al. 1993), which shifted from purportedly fungal-dominated, matted mor humus forms on poorer sites to faunal-dominated, aggregated moder humus forms on richer sites. These contrasting sites under a uniform macroclimate provided an ideal setting for isolating edaphic influences on late-seral EMF communities, and we were able to minimize the possible effects of host diversity (DeBellis et al. 2006) and tree size by sampling a single understory species, subalpine fir (*Abies lasiocarpa* [Hook.] Nutt.), which had naturally regenerated throughout these old-growth forests.

In this study, we report on the relationships between natural gradients in soil productivity and the EMF communities of *A. lasiocarpa*, including diversity estimates, species distribution, and hyphal exploration types (Agerer 2001). We compare our findings with vascular plant diversity–productivity relationships to discuss commonalities in aboveground and belowground community ecology, and discuss some of the possible broader implications of diverse, site-specific EMF communities in boreal landscapes.

Materials and methods

Site descriptions

The southern boreal forest of British Columbia is designated as the Sub-Boreal Spruce Zone (SBS), and is located in the montane landscape of the central interior, within the closed forest portion of the Cordilleran boreal region (Pojar 1996). The SBS has a continental climate characterized by severe, snowy winters and short, warm, moist summers. Upland coniferous forests are comprised of lodgepole pine (Pl) (*Pinus contorta* Dougl. ex Loud), hybrid white spruce (Sx) (*Picea glauca*×*Picea englemanii* [Moench] Voss), and subalpine fir (Bl). Soils are free of permafrost and are predominantly deep blankets of glacial tills with coarse fragments of mixed lithology.

The study sites were located in the moist cold (mc) subzone of the SBS near Smithers, British Columbia, Canada (54°49′N 127°10′W; elevation 522 m). Four site series (represented by climax plant communities corresponding to soil moisture and nutrient regime; Pojar et al. 1987) were sampled to provide a wide range in upland edaphic conditions: (02) xeric and poor Pl–Cladonia; (01) mesic and medium Sx–Huckleberry; (06) subhygric and rich Sx–Oak fern; and (09) subhygric and very rich Sx–Devil's club (Banner et al. 1993). Site series are hereafter

referred to by their nutrient regime and plant association name.

Experimental design

Five blocks were located along a 25-km portion of the McDonnell Forest Service Road (54°40′ to 47′N and 127°16′ to 36′W) at approximately 900 m elevation. Mean annual air temperature of these sites is estimated, based on ClimateBC extrapolation (Spittlehouse 2006) at 2.3°C, with mean annual precipitation of 987 mm (477 mm as snow). One replicate of each plant association was located per block, generally within a radius <1 km (four plant associations × five blocks = 20 plots). We were unable to find a suitable Sx–Devil's club plot at the fourth block, therefore the study was limited to 19 plots. Each plot was 50 m×30 m (0.15 ha) in size. Further descriptions of stand, soil, and vegetation characteristics of the study plots are listed in Kranabetter et al. (2007) and Kranabetter and Simard (2008). Some key site properties published previously are summarized in Tables 1 and 2 and briefly described below.

Site properties

All plots had mixed, late-seral coniferous forests (~180 years) but with differences in relative canopy composition across the gradient; lodgepole pine was the dominant species on nutrient-poor, xeric sites, and was less abundant than subalpine fir or hybrid spruce on moister and richer sites (Table 1). Trees of the canopy had ceased height growth (i.e., reached an asymptote) decades earlier, and we used the height of three co-dominant trees of each species per plot as a measure of site potential (in some of the poor-Cladonia stands only lodgepole pine comprised the overstory). Site index was determined for one co-dominant tree per species per plot using the British Columbia Ministry of Forests Site Tool (Version 3.2B). A fixed area subplot of 0.01 ha was located near the center of each plot to determine stand basal area.

As described in Kranabetter et al. (2007), the mass per hectare (forest floor and mineral soil) of dissolved organic N, NH_4^+, and NO_3^- were determined from a 5-week in situ incubation initiated in early June, 2006. Forest floor F and H horizons were sampled as intact cores, avoiding pure decayed wood, and mineral soils were sampled down to 20 cm with an auger. Mineral soils were sealed in a polyethylene bag within the sample hole, and forest floors were placed on top of this sample in a separate bag. Dissolved organic N (DON) and inorganic N (DIN) was determined colorimetrically using a modified persulfate solution, and forest floor and mineral soil N concentration data was converted to mass per hectare using depth and coarse fragment content values from each plot.

Foliar N concentrations ($N_{\%}$) of understory subalpine fir were determined in mid-September of 2006. The sapling cohort established naturally under the canopy and had been suppressed for some decades on all plots. Current year foliage was clipped from 15 subalpine fir saplings and bulked together to form three subsamples per plot. Foliar samples were oven dried (70°C for 24 h), ground with a Wiley mill, and analyzed for N by dry combustion.

Soil moisture was measured gravimetrically every 3 weeks throughout the summer of 2006 and converted to content (kg ha^{-1}) for the soil profile using the same depth and coarse fragment content values as N determinations. Forest floor pH was measured in water, and total organic phosphorus (P) was determined indirectly with a dry ash and sulfuric acid extraction and an UV–visible spectrophotometer (Varian Inc., Palo Alto, USA).

Ectomycorrhizal fungus assessment

Roots for EMF assessment were sampled June 13–15, 2007 from the understory subalpine fir saplings. Understory saplings are ideal as they limit root sampling to one tree species, and typically host EMF communities comparable to the larger overstory trees (Jonsson et al. 1999; Richard et al. 2005). Soil was removed from around the base of the

Table 1 Stand characteristics and understory *A. lasiocarpa* foliar N concentrations by plant association (means with SE in brackets)

Plant association[a]		Stand height (m)	Site index (m @ 50 years)	Foliar $N_{\%}$ (g kg^{-1})	Basal area (m^2 ha^{-1})	Relative cover (%)		
	n					Pl	Ba	Sx
P—Cladonia	5	21a* (1.4)	12a (1.3)	9.7a (0.12)	33a (3)	80a (3)	15a (4)	5 (2)
M—Huckleberry	5	28b (0.5)	15b (1.4)	11.5b (0.17)	54b (4)	44b (5)	49b (5)	7 (1)
R—Oak fern	5	32c (0.4)	19c (0.3)	12.6c (0.14)	75c (7)	13c (5)	65b (9)	22 (8)
VR—Devil's club	4	36d (0.7)	24d (1.0)	13.6d (0.17)	119d (6)	19c (2)	67b (3)	15 (1)

*Means within columns separated by letters are significantly different ($p<0.05$)

[a] Soil nutrient regimes *P* poor, *M* medium, *R* rich, *VR* very rich

Table 2 Soil nitrogen indices (dissolved organic N, ammonium, and nitrate after a 5-week in situ incubation) and selected properties by plant association (means with SE in brackets)

Plant association[a]	*n*	DON (kg ha^{-1})	NH_4^+ (kg ha^{-1})	NO_3^- (kg ha^{-1})	Mineral soil pH (H_2O)	Forest floor pH (H_2O)	Soil moisture (kg m^{-2})	Organic P (kg ha^{-1})
P—Cladonia	5	16.7a (2.7)	0.9a (0.2)	0a	4.8 (0.06)	4.0a (0.05)	13.4a (1.2)	137a (8)
M—Huckleberry	5	27.1b (1.6)	3.2b (1.0)	0a	4.6 (0.05)	4.1a (0.07)	18.7a (1.5)	179ab (25)
R—Oak fern	5	33.1b (1.4)	7.5c (1.0)	0.2b (0.1)	5.2 (0.20)	4.7b (0.15)	29.3b (2.1)	246b (24)
VR—Devil's club	4	32.0b (3.3)	9.2c (3.6)	5.5c (3.3)	5.3 (0.09)	4.8b (0.14)	27.6b (2.0)	446c (39)

*Means within columns separated by letters are significantly different ($p<0.05$)
[a] Soil nutrient regimes *P* poor, *M* medium, *R* rich, *VR* very rich

sapling to reveal the larger, radiating structural roots (5–10 mm in diameter). Three of these roots were clipped and gently excavated from the surrounding soil as completely as possible. Roots were positioned primarily above or along the humus–mineral soil interface and occasionally through buried wood, so feeder roots were extracted from all substrate types to some degree. Five healthy, widely spaced saplings (minimum 10 m apart) were selected per plot, for a total of 95 (5×19 plots) saplings in the study. The root systems were wrapped in moss to keep the root tips fresh, placed into a plastic bag, and returned to the laboratory. Sixty root systems were refrigerated and examined immediately, while the remaining 35 root systems were frozen until the fall before completing the ectomycorrhizal assessment.

The three root segments from each sapling were washed gently in warm water to remove most of the soil and organic debris. Once all surface debris was removed, the clean roots were cut into approximately 2.5-cm-long sections and placed in a glass pan filled with water. Sections were continuously mixed and individual segments randomly selected to determine the number of root tips colonized by each EMF morphotype. Successive root sections were examined until 200 root tips had been classified from each of the saplings. EMF colonization rates were virtually 100%, and in rare cases a root segment was discarded and replaced if the mantle was too young and undeveloped to identify so that a complete census of 200 colonized root tips could be made. The total number of fine roots assessed for the study was 19,000 (95 saplings×200 root tips per sapling).

Each root tip was examined stereoscopically (10× to 40× magnification) for features such as color, shape, size, and texture of the root tip as well as emanating elements, if present. The root tips were examined with a compound microscope at 1,000× magnification for characteristics of the mantle layers and emanating elements such as mantle type, ornamentation, cell contents, clamp frequency, and lengths and widths of hyphal cells. Slides were prepared using either mantle squashes or mantle peels if fungal layers of the mantle were exceptionally thick. When necessary, the root tips were stained with either 0.1% (*w*/*v*) aqueous toluidine blue O, 10% (*w*/*v*) KOH, or Meltzer's reagent to emphasize the mantle features. We named the morphotype if it matched descriptions of species published by the British Columbia Ectomycorrhizal Research Network (2007). In addition, we characterized the hyphal exploration type of each morphotype based on Agerer (2001): 'contact' types had smooth mantles and no rhizomorphs; 'short' types had emanating hyphae with no rhizomorphs; 'fringe' types had long emanating hyphae with diffuse rhizomorphs; 'mat' types had short emanating hyphae with cottony rhizomorphs; 'smooth' types had few or no emanating hyphae and undifferentiated rhizomorphs; and 'long' types had smooth mantles and highly differentiated rhizomorphs.

Molecular techniques

DNA information was used to clarify the taxonomy of distinct but unknown EMF morphotypes and to distinguish between highly similar morphotypes. This latter objective was especially important for *Cortinarius*, as most of these species share a similar morphology (bent to tortuous root tips with thick, white emanating hyphae [4–5 μm in diameter with large clamps] and few other notable features).

Five to ten root tips were collected from 96 fungal colonies of interest (a cluster of root tips colonized by the same EMF morphotype on an individual sapling) and frozen for subsequent DNA extraction and PCR amplification of the fungal ITS region of nuclear rDNA. Samples of one to three tips were placed into a fast prep extraction tube containing AP1 solution of the DNeasy 96 Plant Kit (Qiagen, Mississauga, Canada). The tips were pulverized with a ceramic bead in a FastPrep (FP120) high-speed shaker (Thermosavant, Holbrook, USA). After centrifuging briefly, the supernatant was transferred into wells of a 96-

well plate supplied by the DNeasy Plant Kit. As per the instructions of the DNeasy Plant Kit, 130 μl of the AP2 buffer was added to each well and shaken for 15 s, then stored at −20°C for 10 min followed by centrifuging at 4,000 rpm for 10 min. Six hundred microliters of the AP3/E solution was added to 400 μl supernatant and the resultant solution was shaken vigorously for 15 s, centrifuged to 3,000 rpm, and then immediately stopped. One milliliter of each sample was added and vacuumed from each well of a new DNeasy plate. Four hundred microliters of the AW buffer was added and vacuumed from each well after which this step was repeated, and the plate was dried at 40°C. The DNeasy plate containing the DNA was eluted into elution tubes by adding 100 μl of the AE buffer, waiting 1 min and then vacuuming, after which this step was repeated. The DNeasy plate was centrifuged for 3 min at 2,000 rpm to remove final amounts of DNA. The resultant genomic DNA was stored at −20°C. Primer pairs used in PCR amplifications were either ITS1F-ITS4B or NSI1-NLC2. Samples were cycle sequenced using the Big Dye Terminator Kit (Applied Biosystems, Foster City USA) and the primer set ITS1f and ITS4. Sequencing was performed on a 3,130×1 capillary sequencer (Applied Biosystems). Forward and reverse sequences were aligned and manually corrected in Sequencher 4.2 (GeneCodes, Ann Arbor, MI, USA). Sequences were BLAST searched (Altschul et al. 1997) against the GenBank database to suggest taxonomic affinities of the samples.

Data analysis

EMF species diversity was described in three ways, following Newmaster et al. (2003): species richness per sapling, species richness by plot (alpha diversity, α), and cumulative species richness by plant association (gamma diversity, γ). Shannon's diversity index for the EMF community of each plot (five saplings combined) was determined using PC-ORD 5.0 (McCune and Grace 2002).

The study was organized in a randomized incomplete block design. Species richness and hyphal exploration type abundance was tested among plant associations using Proc Mixed in SAS (SAS Inc 2004) with block and block interactions set as random factors. Residuals from the analyses were examined for normal distributions and found to meet the assumptions of equal variance. Significant differences between least square means of each plant association were tested using pairwise t tests at a significance level of 0.05. The general linear model procedure in SAS using Type 1 Sums of Squares was used to test linear and curvilinear correlations between plot means of dependent and independent variables (n=19). No significant effect of block or block×treatment interactions was found in any of the correlations. We chose a significance p value of 0.010 for correlations of EMF species abundance because of the inherently high variation in species occurrence and scale of sampling.

A comparison of EMF fungal communities among plots was undertaken by non-metric multi-dimensional scaling (NMS), using the relative Sorenson measure for species abundance. Computations were undertaken with PC-ORD 5.0 software, using the random starting configurations (McCune and Grace 2002). The ordination of axes was tested against plot soil measures using Pearson and Kendall correlations and the ordination graph rotated to the variable with the strongest correlation. Separation of EMF communities by plant association was tested in pairwise comparisons using the multi-response permutation procedure (MRPP) with the Sorenson (Bray–Curtis) distance measure (presence/absence) (McCune and Grace 2002). EMF community similarity based on species abundance (% root colonized) was determined by percentage similarity (PS) (Pielou 1984).

Results

Initial morphotyping distinguished 63 EMF taxa, and 75% of the distinct but unknown morphotypes were identified to the closest aligned species through ITS rDNA analysis (Appendix). We were able to separate the *Cortinarius* colonies into 24 species using ITS rDNA as well; a small number of inconclusive results were designated as *Cortinarius* sp. A decision was made to lump together a few infrequent but similar morphotypes (likely from the Thelephoraceae family) when we were unable to confirm unique species identification or if consistent separation of morphotypes was not possible. With these adjustments, the total number of taxa used in the statistical analysis was 74 species. This included, in part, a dark septate fungus (MRA), four species of *Piloderma*, seven of *Tomentella* and *Pseudotomentella*, eight of *Russula*, 27 of *Cortinarius*, two of *Lactarius*, one of *Tricholoma*, and a variety of unknown fungi (Appendix; a partial list of the more common EMF taxa is presented by plant association in Table 2). We lacked the resources to sequence every morphotype on every sapling in this survey, and so certain fungi treated as a single species, such as *Cenoccocum geophilum*, may represent species complexes (Jany et al. 2002).

The number of EMF species per sapling ranged from one to 14, and average richness per sapling was significantly lower (p=0.006) on poor-Cladonia sites compared to the other plant associations (Table 3). A similar trend was found in α diversity of the plots, with approximately 20 species on average for the medium to very rich plant associations (Table 3). The extent of EMF α diversity

Table 3 Diversity measures (means with SE in brackets) and abundance (mean % root colonization) for the more frequent ectomycorrhizal fungi grouped by plant association (% frequency by sapling; 25 in total for poor, medium and rich plant associations, 20 for very rich)

	Poor—Cladonia (n=5)	Medium—Huckleberry (n=5)	Rich—Oak fern (n=5)	Very Rich—Devil's club (n=4)
Richness per sapling	6.0a* (0.3)	7.2b (0.4)	7.7b (0.4)	7.5b (0.4)
α diversity (per plot)	15.6a (0.8)	19.6b (1.0)	20.2b (1.2)	20.8b (0.9)
Shannon's index (per plot)	2.18 (0.15)	2.36 (0.08)	2.43 (0.08)	2.50 (0.03)
γ diversity (all replicates)	33	34	41	41
Percent root colonization (% frequency)				
Cenococcum geophilum	9.6 (80)	16.6 (92)	13.7 (100)	9.1 (65)
MRA	25.6 (72)	20.9 (88)	9.6 (56)	1.0 (15)
Unknown fungus VI	10.1 (44)	4.0 (36)	4.6 (36)	1.8 (20)
Unknown fungus VIII	8.7 (56)	3.3 (36)	3.4 (28)	3.8 (40)
Amphinema byssoides	0.2 (4)	2.2 (16)	4.7 (32)	13.4 (55)
Laccaria laccata	1.8 (8)	2.8 (20)	5.4 (52)	9.9 (65)
Piloderma fallax	5.6 (60)	10.6 (68)	6.4 (60)	1.5 (25)
Piloderma I	2.0 (36)	5.0 (40)	11.5 (60)	9.5 (75)
Piloderma II	0	0	1.7 (12)	3.6 (15)
Piloderma III	1.2 (16)	0.7 (8)	0	0
Cortinarius cf. *semisanguineus*	1.5 (32)	2.4 (40)	0.6 (8)	0
Cortinarius neofurvolaesus	2.2 (8)	0	0	0
Cortinarius cinnamomeus	0	1.2 (24)	0.9 (20)	0
Cortinarius III	0	0.3 (4)	0	3.6 (5)
Cortinarius hemictrichus	0	0.7 (16)	1.1 (24)	2.2 (20)
Inocybe lanuginosa-like	0	0.5 (4)	0.5 (12)	1.1 (10)
Inocybe I	0	0	0.3 (4)	1.5 (10)
Leccinum aurantiacum	1.1 (12)	0	0	0
Russula decolorans	4.7 (8)	1.6 (8)	0	0
Russula III	2.2 (4)	0	0	0
Russula bicolor	0	0.4 (12)	2.2 (36)	2.0 (20)
Russula I	0	0	1.2 (16)	3.0 (10)
Russula II	0	0	0	3.7 (15)
Thaxterogaster cf. *pinguis*	0	5.3 (20)	3.8 (24)	1.8 (15)
Sarcodon sp.	7.9 (24)	0	0	0
Tomentella cf. *stuposa*	0	0	0	8.1 (55)
Cortinarius XII	0	0.7 (4)	3.0 (16)	1.7 (5)
Unknown fungus II	0.2 (4)	2.8 (24)	1.7 (12)	0
Unknown fungus V	0	4.0 (28)	5.8 (24)	0.4 (5)
Unknown fungus VI	0.9 (8)	3.4 (24)	2.7 (16)	0

*Means within columns (diversity measures only) separated by letters are significantly different (p<0.05)

increased asymptotically with soil fertility in regression analysis, as demonstrated by the positive curvilinear correlation with foliar $N_{\%}$ of the saplings (Fig. 1). Removing one outlier contributed by a rich-Oak fern site improved the precision of the equation (r^2 from 0.59 to 0.71) but had little effect on the significance or shape of the curve. Shannon's diversity index averaged 2.36 overall (Table 3), and we were unable to detect significant differences among plant associations (p=0.153). Plant association (γ) diversity peaked at 41 species on rich-Oak fern and very rich-Devil's club sites (Table 3), equal to an approximately 20% increase over poor-Cladonia and medium-Huckleberry sites.

The EMF communities showed a progressive separation by plant association in the NMS analysis that followed the productivity rankings (Fig. 2; the proportion of variance along axes 1 and 2 were 0.577 and 0.226, respectively, for a cumulative r^2 of 0.803). Pearson and Kendall correlations were most significant between axis 1 and soil N indices, including inorganic N mass (r^2=0.803) and DIN:DON ratio (r^2=0.758). Axis 2 was best defined by the geochemistry variables of exchangeable K (r^2= 0.391) and mineral soil pH (r^2=0.200). Asymptotic stand height was also a significant correlate for axis 1 (r^2= 0.715), although all site potential indices covary strongly

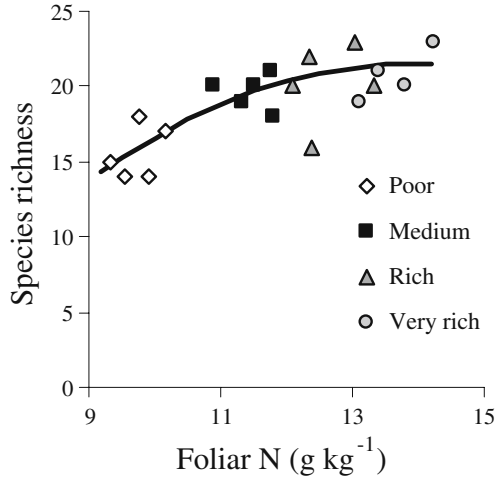

Fig. 1 EMF species richness per plot (α diversity) in correlation with foliar N concentration of the *A. lasiocarpa* understory (n=18, one rich-Oak fern plot not included). Richness=−41.8+9.14 (foliar $N_{\%}$)−0.33(foliar $N_{\%}$)^2; $p<0.001$; $r^2=0.71$

with soil N indices and foliar $N_{\%}$ (Tables 1 and 2; Kranabetter and Simard 2008).

Significant differences in EMF community composition (presence/absence) were detected in MRPP comparisons of species assemblages between poor, medium, and very rich sites (Table 4). PS analysis also revealed an increasing dissimilarity in EMF fungal distribution and abundance with soil fertility, equal to a 24% overlap in EMF communities between the extreme contrasts in plant associations (Table 4). An intermediate degree of shared EMF species was found between medium-Huckleberry and rich-Oak fern sites (Fig. 2, Table 4), likely reflecting the consistency in forest floor N supply between these two plant associations (N mineralization potential of 624 and 656 mg kg^{-1}, respectively; Kranabetter et al. 2007). Very few EMF species were evenly distributed across plant associations, and some of the more common EMF species had significant trends ($p<0.010$) in abundance in relation to soil fertility. This was demonstrated for six EMF species, and included parabolic, negative linear, and positive exponential curves in correlations with foliar $N_{\%}$ (Fig. 3).

There were few generalizations that could be drawn on the distribution of EMF genera. *Inocybe* and *Tomentella* species tended to favor richer soils, but other speciose genera such as *Cortinarius* and *Russula* had individual species better adapted to either end of the productivity spectrum (Table 3). The distribution of EMF by exploration type was quite consistent among plant associations, averaging seven contact, 11 short-distance, 14 medium-fringe, two medium-mat, and three medium-smooth species per plot. The abundance of three exploration types changed significantly with plant association (Fig. 4); short exploration fungi declined on the rich-Oak fern and very rich-Devil's club sites ($p=0.028$), as medium-fringe and medium-smooth fungi increased ($p=0.033$ and $p=0.037$, respectively).

Discussion

The significant and consistent changes in distribution and abundance of 74 EMF species demonstrated a high degree of community specialization along these gradations in soil fertility. It is difficult, however, to isolate the exact causes of EMF species distribution because of the number of covarying site properties which may influence EMF communities, including N and P availability, soil moisture, and pH. Nitrogen is a useful focus for analysis because its availability in boreal ecosystems

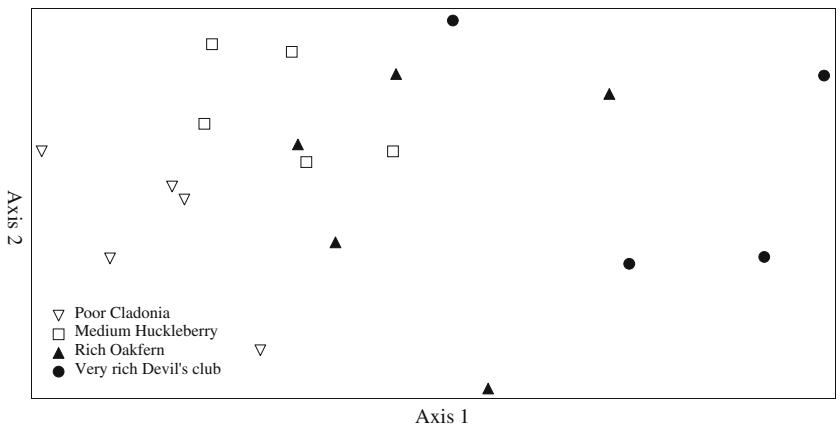

Fig. 2 Non-metric multidimensional scaling analysis of EMF communities among the 19 plots (based on the abundance of 74 species), rotated to maximize correlation with inorganic N mass on axis 1

Table 4 Matrix of ectomycorrhizal fungal community similarity between plant associations by MRPP (p values based on presence/absence of species) and, in brackets, percentage community similarity (based on mean % root colonization by species)

M—Huckleberry	0.003 (56)		
R—Oak fern	0.005 (42)	0.340 (66)	
VR—Devil's club	0.004 (24)	0.007 (35)	0.126 (52)
	Poor—Cladonia	Medium—Huckleberry	Rich—Oak fern

integrates underlying soil moisture and geochemical drivers well, and correlates strongly with forest productivity, both as soil N indices and foliar N concentration (Kranabetter and Simard 2008). Given the strong evidence for direct effects of N on EMF physiology (e.g., Arnebrant 1994) it was most relevant to our objectives to examine natural ranges in N availability, but we recognize other ecosystem attributes could be influential on EMF and deserve consideration. For example, an effort was made to equalize the potential effect of neighbors (Hubert and Gehring 2008) by choosing sites with mixed stands of pine, fir, and spruce, although it was not possible to find an equal distribution of the three conifer species across all sites (Table 1). Perhaps then the EMF community parameters would differ under pure *Abies lasiocarpa* forests to some degree, but we would argue a high degree of EMF community specialization with soil properties would still exist. The differences in overstory tree size and rates of C fixation were controlled by sampling a

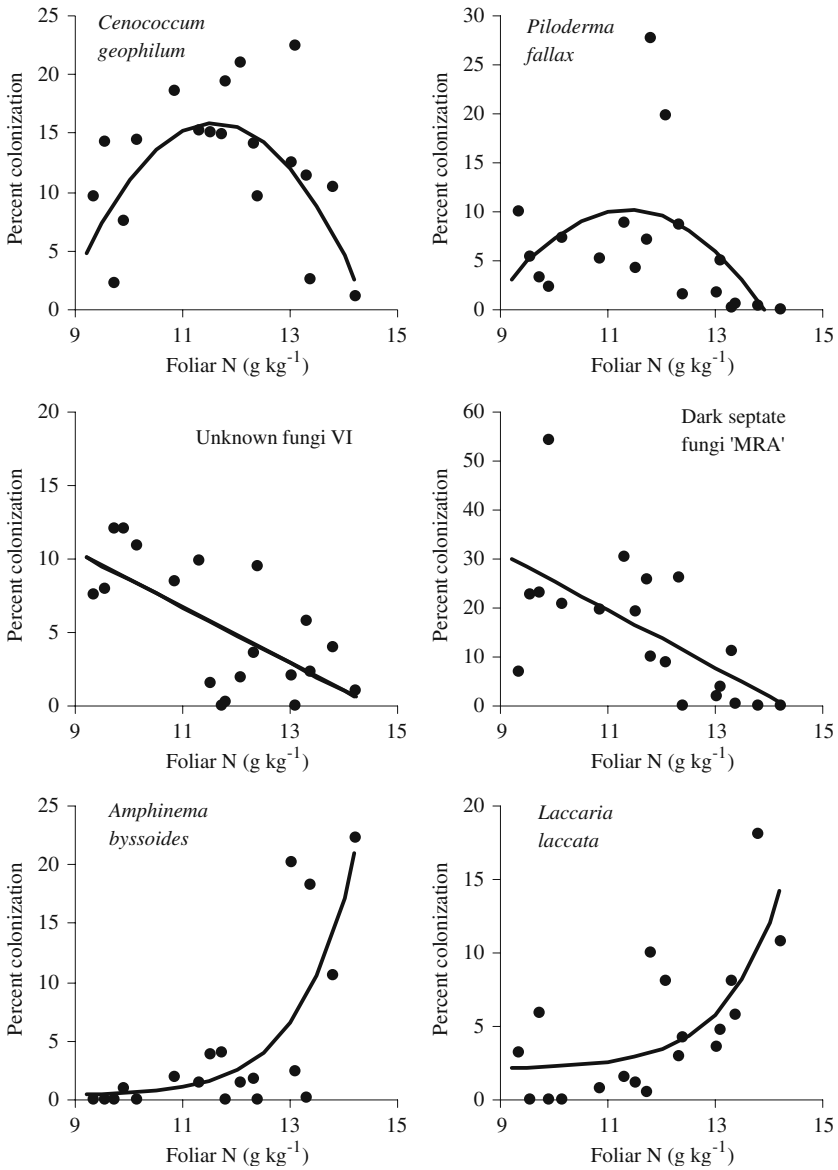

Fig. 3 Abundance of six EMF species in correlation with foliar N concentration of *A. lasiocarpa* *C. geophilum*=−245+45.2 (Foliar $N_{\%}$)−1.9 (Foliar $N_{\%}$)^2; p=0.010, r^2=0.43; *P. fallax*=−191+35.4 (Foliar $N_{\%}$)−1.6 (Foliar $N_{\%}$)^2; p=0.090, r^2=0.26; MRA=84−5.8 (Foliar $N_{\%}$); p=0.004, r^2=0.40; unknown fungi VI=27.6−1.9 (Foliar $N_{\%}$); p=0.002, r^2=0.45; *A. byssoides*=0.28+0.000014e$^{(\text{Foliar N\%})}$; p=0.001, r^2=0.59; *L. laccata*=2.1+0.0000083e$^{(\text{Foliar N\%})}$; p=0.003, r^2=0.50

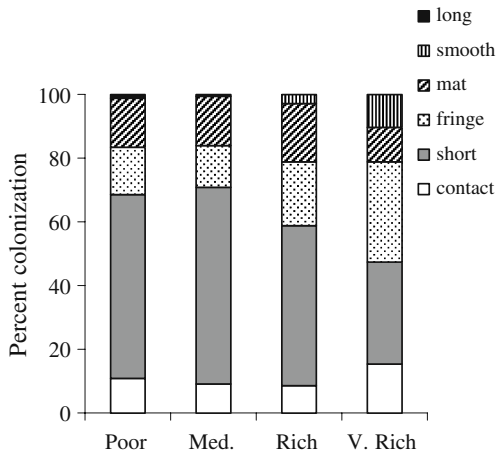

Fig. 4 Hyphal exploration types of EMF as a mean percent of root colonization grouped by plant association (n=5 for poor, medium, and rich plant associations; 4 for very rich)

comparable cohort of suppressed advanced regeneration (~1.5 m in height) on all plots; in any case, it is uncertain how significant photosynthesis rates might be since there is little evidence for differences in EMF communities between illuminated overstory and shaded understory trees (Jonsson et al. 1999; Richard et al. 2005).

The increase in EMF α diversity with foliar $N_{\%}$ demonstrated that many of these EMF species were well suited to soils with high rates of N mineralization, at least within the context of these cool, moderately productive boreal landscapes. A hump-backed or unimodal distribution of plant diversity with soil fertility is often proposed (and widely debated) by ecologists, where relatively few plant species are successful on both the most stressful and competitive sites (Mittlebach et al. 2001). Some parallels can be drawn to this EMF community since there was a reduction in the number of species on the driest, N-poor soils, but no corresponding reduction on the most productive sites. Species such as *A. byssoides*, *L. laccata*, and *T. stuposa* were gaining in dominance, but the rates of N mineralization and nitrification on very rich-Devil's club sites were perhaps never high enough to allow more complete competitive success. For this reason, we suspect the peak in EMF diversity coincided with the more heterogeneous supply of all three N forms (amino acids, NH_4^+, and NO_3^-) associated with rich and very rich soils (Kranabetter et al. 2007), and we are unaware of any (ultra-rich) ecosystems supplied entirely by inorganic N in these boreal landscapes. In addition, productive ecosystems have a component of poor microsites, such as buried wood, that would contribute to niche diversity and species richness (Buée et al. 2007; Iwański and Radawska 2007). Positive productivity–species richness relationships such as these are not entirely uncommon among plant or animal taxa, especially when compared within a community type or over a limited productivity range (Mittlebach et al. 2001).

The increase in EMF diversity and medium-distance exploration types with soil fertility were largely at odds with results reported from N fertilization or N deposition studies (Lilleskov et al. 2002; Nilsson and Wallander 2003). Toljander et al. (2006) noted a similar discrepancy, and suggested that the range of N concentrations among natural soils is of a much smaller magnitude than those experimentally applied, resulting in more dramatic effects of N fertilizer on EMF communities. For example, the anthropogenic fertility gradient for *Picea glauca* (Lilleskov et al. 2002) had foliar N concentrations of 13.9 g kg^{-1} under the lowest N inputs, which would actually be comparable to our richest sites (13.6 g kg^{-1}). Certain tree species, especially of *Pinus*, may be better adapted to poor soils and organic N forms and consequently respond differently than *Abies* to inorganic N availability (Berch et al. 2006; Parrent et al. 2006; Taniguchi et al. 2007). Another consideration is that EMF evolved with niches that occur naturally in forests such as the high soil moisture and inorganic N availability found together on rich-Oak fern and very rich-Devil's club sites (Kranabetter and Simard 2008). Perhaps then high amounts of N deposition on mesic sites would be unsuitable for eutrophic EMF species that cannot tolerate soil droughtiness or higher acidity. It is very likely that various soil properties (nitrogen, moisture, pH, etc.) must be aligned to create suitable habitat (Trudell and Edmonds 2004), and any perturbations to forest ecosystems resulting in habitats with no natural analogue could be detrimental to EMF.

Among these diverse communities were EMF species which varied in abundance but were present at least to some degree on all site types (e.g., *C. geophilum*, *Piloderma* I, unknown fungus VIII). This wide habitat distribution ('multi-site') could be a significant contribution to the resiliency of these forest ecosystems as it would allow quick responses to any positive or negative changes in soil resource availability (resiliency defined as the capacity to absorb disturbances without undergoing change to a fundamentally different state; Drever et al. 2006). An example is the flush of inorganic N commonly occurring after forest disturbances, and it is likely of some importance that many of these multi-site EMF species are also multi-seral and multi-host fungi, able to persist and thrive in regenerating stands with many tree species (Kranabetter 2004). The capacity of EMF to buffer disturbances, in a resilience context, might also include severe drought events (Swaty et al. 2004) or more gradual but significant climatic trends (e.g., Pacific Decadal

Oscillation) that could affect soil processes and nutrient availability. Along with generalist fungi, there were also EMF species more limited in distribution (e.g., *R. decolorans*, unknown fungi VI, *T. stuposa*), that may be well adapted to specific edaphic niches and contribute to increased utilization of soil resources. Ectomycorrhizal fungus communities may have a degree of functional similarity, as with many soil biota, but certainly a mix of species attributes (multi-site and site-specific species, multi-seral and late-seral species, multi-host and host-specific species) should insure resiliency and sustain productivity in a stressful, dynamic, and unpredictable forest environment (Perry and Amaranthus 1997).

It is perhaps not surprising there were only minor trends in the distribution of fungal exploration types with soil fertility in comparison to N fertilizer treatments given the relatively subtle shift in N amounts and forms. The consistent mix of mantle types could reflect high functional diversity in response to the heterogeneity of microsites and resources found throughout the fertility gradient (Baier et al. 2006). Presumably EMF would contribute significantly to microbial biomass across the entire productivity gradient, with some effect of rooting density, while shifts in ericoid and arbuscular fungi would correspond to the distribution of understory plants (Nilsson et al. 2005). The visual perception of EMF abundance, as a characteristic of humus forms (Green et al. 1993), is more likely a reflection of shifts in EMF communities on sites such as these because conspicuous mat-forming fungi, especially bright yellow *P. fallax*, declined as dark-colored *Tomentella* spp. gained in abundance on the richest sites. Categorizing more diverse fungal genera such as *Cortinarius* or *Russula* into habitat types would be an oversimplification as these species occupied all manner of niches, similar to the patterns in genera distribution with forest succession (Kranabetter et al. 2005; Twieg et al. 2007). Tracking the full complement of EMF species through root sampling would be exceedingly difficult (Taylor 2002), however, and sporocarp surveys may help define the distribution of the more infrequent fungi. Presumably EMF communities in older forests such as these have little change in dominant species composition over time (Izzo et al. 2005), but possible seasonal effects might also be of interest in studies of soil abiotic–biotic relationships (Koide et al. 2007).

Other than poor-Cladonia sites, the difference in α diversity or Shannon's diversity index among plant associations was quite insignificant compared to the more profound shifts in EMF species distribution and community composition. For this reason, we would suggest diversity parameters are not always the most relevant variable compared to the identity and abundance of the EMF species themselves in evaluating forest processes (Wallenda et al. 2000; Dahlberg 2001). Likewise, it is possible that controlled studies with ad hoc EMF species assemblages could draw incongruous conclusions if ill-suited EMF species were selected for the experimental soil conditions. For example, greenhouse studies of tree nutrition and N forms do not always account for EMF species composition (e.g., Bennett and Prescott 2004), which is understandable given the inability to recreate such specialized and complex EMF communities, but this simplification could affect the outcome of these experiments. Plant ecologists are acutely aware of hidden treatment effects in experimental manipulation of plant communities (Huston 1997), and we would caution that similar confounding effects of EMF species need to be considered in testing of tree–soil interactions.

In conclusion, the results suggest that EMF species distribution across landscapes, like many vascular and non-vascular forest plants, is largely defined by adaptation and competition for niches related to stress tolerance (e.g., drought, soil acidity) and resource availability (especially organic N, NH_4^+, and NO_3^-) in soils (Dickie et al. 2002; Koide et al. 2005). The significance of such extensive EMF β diversity with a single tree host, especially in contrast to the much greater aboveground diversity of plants with arbuscular fungi (Allen et al. 1995), is worth further consideration. A reasonable conjecture, from both this and similar results (Gehring et al. 1998; Toljander et al. 2006; Robertson et al. 2006), is that the wide ecological amplitude of relatively few tree species across vast boreal and temperate landscapes would depend to some degree on partnerships with well-adapted EMF fungal assemblages. Consequently, the simplification of EMF communities through anthropogenic activities might hamper the survival of conifers on stressful sites, impede their ability to compete with arbuscular plants on productive sites, or reduce the stability of forests in dynamic and unpredictable environments. These hypotheses are not easily validated, but present some of the possible long-term risks to consider in the evaluation of stressors (intensive forestry, atmospheric pollution, invasive species, and climate change) on forest ecosystems.

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Appendix

Table 5 List of morphotypes with successful ITS rDNA analysis

Provisional name	Closest GenBank match	% Match	Comment
Mycelium radicis atrovirens (MRA)	DQ481971	638/648 (98%)	Uncultured ectomycorrhiza
Cortinarius cf. *anomalus*	EU525957	575/577 (99%)	
Cortinarius boulderensis	DQ499466	630/636 (99%)	
Cortinarius calopus	FJ039571	492/494 (99%)	
Cortinarius canabarba	FJ039562	545/549 (99%)	
Cortinarius clandestinus	FJ039583	587/587 (100%)	
Cortinarius firmus	AF389163	544/544 (100%)	
Cortinarius hemitrichus	AY669680	498/502 (99%)	
Cortinarius malicoria	DQ481917	715/721 (99%)	
Cortinarius neofurvolaesus	DQ140002	478/479 (99%)	
Cortinarius saturnius	FJ039551	664/673 (98%)	
Cortinarius cf. *semisanguineus*	DQ481909	580/595 (97%)	Morphotype matched except color was light pink
Cortinarius triformis	FJ039573	536/540 (99%)	
Cortinarius vibratilis	EU821696	658/663 (99%)	
Cortinarius I	AY669687	342/348 (98%)	*Cortinarius umbilicatus*
Cortinarius II	AJ889975	370/377 (98%)	*Cortinarius praestigosus*
Cortinarius III	FJ039675	249/257 (96%)	*Cortinarius paragaudis*
Cortinarius IV	DQ102683	428/446 (95%)	*Cortinarius* cf. *saniosus*
Cortinarius V	EF218763	511/516 (99%)	Uncultured (*Cortinarius*)
Cortinarius VI	AJ438981	578/599 (96%)	*Cortinarius obtusus*
Cortinarius VII	DQ481963	549/552 (99%)	Uncultured (*Cortinarius*)
Cortinarius VIII	AF325590	487/504 (96%)	*Cortinarius brunneus*
Cortinarius IX	DQ481959	635/648 (97%)	Uncultured cf. Dermocybe
Cortinarius X	EF218758	444/446 (99%)	Uncultured (*Cortinarius*)
Cortinarius XI	EU693242	509/520 (97%)	*Cortinarius testaceofolius*
Cortinarius XII	EF077497	321/328 (97%)	Uncultured (*Cortinarius*)
Unknown fungi I	FJ152525	371/392 (94%)	Uncultured (Helotiales)
Unknown fungi II	DQ481700	438/439 (99%)	Uncultured ectomycorrhiza
Unknown fungi III	DQ481971	486/488 (99%)	Uncultured ectomycorrhiza
Unknown fungi IV	AY825525	684/699 (97%)	Uncultured Thelephoraceae
Unknown fungi V	EU057086	583/590 (98%)	Uncultured Thelephoraceae
Unknown fungi VI	AY822734	614/623 (98%)	Uncultured ectomycorrhiza
Unknown fungi VII	AY702742	271/279 (97%)	Uncultured ectomycorrhiza
Unknown fungi VIII	AY394895	619/670 (92%)	Uncultured ectomycorrhiza
Inocybe I	DQ093854	396/413 (95%)	*Inocybe geophylla*
Lactarius rufus	EF685089	498/498 (100%)	
Piloderma fallax	DQ658864	406/406 (100%)	
Piloderma I 'Green globs'	EU057111	388/420 (92%)	Uncultured *Piloderma*
Piloderma II 'Glass shards'	DQ474735	521/538 (96%)	Uncultured *Piloderma*
Piloderma III 'Peaches'	DQ377372	504/544 (92%)	Uncultured *Piloderma*
Russula I	AY061685	421/435 (96%)	*Russula laricina*
Russula II	EF433961	713/725 (98%)	Uncultured *Russula*
Russula III	AB211253	432/442 (97%)	Uncultured *Russula*
Tomentella cf. *stuposa*	AF272902	439/440 (99%)	*Tomentella stuposa*
Tomentella I	AJ534911	625/649 (96%)	*Tomentella* sp. O53
Tomentella II	DQ974777	491/517 (94%)	*Tomentella lateritia*
Tomentella III	TSU83470	612/617 (99%)	Thelephoraceae 'Taylor #6'
Pseudotomentella humicola	AM490945	555/559 (99%)	
Pseudotomentella sp.	AJ893352	611/617 (99%)	Uncultured *Pseudotomentella*
Thaxterogaster cf. *pinguis*	DQ328112	357/364 (98%)	*Thaxterogaster pinguis*
Sarcodon sp.	AF103896	649/672 (96%)	*Sarcodon squamosus*
Tricholoma sp.	AY656987	424/425 (99%)	Uncultured *Tricholoma*

Note: additional species recognized through morphotype characters included *Cenococcum geophilum*, *Amphinema byssoides*, *Cortinarius cinnamomeus*, *Laccaria laccata*, *Leccinum aurantiacum*, *Lactarius kaufmanii*, *Rozites caperata*, *Russula aeruginea*, *Russula bicolor*, and *Russula decolorans* (British Columbia Ectomycorrhizal Research Network 2007). A further 11 taxa were characterized as morphotypically distinct but were unsuccessful in DNA sequencing. Species identity was assumed when the match with GenBank was 98% or better at >450 base pairs, otherwise the closest matching species name was noted under comments

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