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Title: Developing indicators of soil productivity, function and biodiversity through soil biotic communities.

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Abstract

Potential indicator species for forest management were explored in a series of studies of old-growth boreal stands encompassing a range of inherent site productivity. The biota surveyed included terrestrial cryptogams, ectomycorrhizal fungi on roots of *A. lasiocarpa*, epigeous ectomycorrhizal mushrooms, mesofauna of the forest floor (mites and springtails) and macrofauna (beetles, ants, and spiders). Manuscripts on the cryptogams and ectomycorrhizal fungi are completed, and final resolution of a few remaining faunal taxa is almost complete. Some interim conclusions on the overall patterns of biodiversity are presented here. The number of species per plot (0.15 ha) ranged from 145 to 205 (vascular plants, cryptogams, ectomycorrhizal fungi, springtails, spiders and ants), which represents about 1/3rd the total diversity of this landscape (607 species; not including beetles, mites). Total species richness by plot increased by approx. 33% and then plateaued with site productivity, suggesting that greater amounts of soil moisture, nutrients and organic matter allowed for the development of a richer community of soil organisms. Beta diversity, which is the turnover of species across a landscape, averaged 31% by plot, meaning any one individual plot had approx. 30% of the total species found over the 19 plots. The uniqueness of species assemblages by site series was moderate, and so, for example, preserving mesic sites alone would provide habitat for about 50% of the total landscape organisms. Capturing a range of dry to moist upland sites as oldgrowth management areas or wildlife reserves would therefore potentially double the total number of species within the conservation areas. Our results might also be considered in monitoring programs for late-seral species, which generally use space for time substitution as an experimental design. Such an approach would be

less dependent on site for soil fauna, as the majority of these species were ubiquitous, whereas cryptogams and ectomycorrhizal fungi could be confounded by edaphic effects on species assemblages. The benefit of this finer site association, however, is that these species could provide more sensitive indicators for ongoing changes in soil fertility under forest management. Overall we found a large source of biodiversity associated with forest soils and substrates (6 x the diversity of the vascular plants), and a large pool of potential species for environmental monitoring. Indicators could include those species representing oligotrophic, mesotrophic or eutrophic soil conditions, and functional organization such as the balance of rare versus common species on the landscape.

1. Introduction

Indicator species of migratory songbirds, raptors, small mammals, epiphytic lichens and wood-decay fungi have been touted as useful fine-filter tools in ecosystem monitoring (Nilsson et al. 2001; Kremsater et al. 2003). Soil indicators of sustainability are currently limited to soil disturbance surveys and reductions in net areas to be reforested (Montréal Process 1995), and many soil biota, with the exception of select arthropods, have yet to be thoroughly assessed for suitability as indicators (Thompson 2006). More biologically-based indicators of soil fauna, ectomycorrhizal fungi, or terrestrial nonvascular plants could provide more sensitive criteria of management practices related to soil function, productivity, and biodiversity.

A logical step in the development of soil indicators is a better characterization of biotic communities across sites representing full gradients in ecosystem productivity. Species that are limited in distribution to poor ecosystems, for example, might serve as biological indicators of site-degrading forest practices. Better information on species distribution and community composition is also essential in providing unambiguous indicators across spatial scales. For example, twelve mushroom species limited to late-seral stands in the ICH were only tested on submesic ecosystems (Kranabetter et al. 2005), and might not be valid for richer ecosystems in that landscape. We also have very little information on the natural range in variability for community measures in soils, such as average species richness or relative eveness across sites. Targets or criteria extrapolated from studies limited to mesic sites could lead to poor assessments of management impacts on soils across variable landscapes.

In this project we report on the soil biotic communities associated with replicated productivity gradients of upland southern boreal forests in British Columbia (Kranabetter et al. 2007). The plots were situated in close proximity and elevation, with old-growth stands of *Pinus contorta* (Dougl. ex Loud), *Abies lasiocarpa* ([Hook.] Nutt.), and *Picea glauca* x *englemanni* ([Moench] Voss) at almost every site that created an ideal study design to minimize the effects of climate, stand age, and tree species composition on relationships between soil fertility and biotic communities. Our studies include terrestrial cryptogams, ectomycorrhizal fungi on roots of *A. lasiocarpa*, epigeous ectomycorrhizal

mushrooms, mesofauna of the forest floor (mites and springtails) and macrofauna (beetles, ants, and spiders).

These studies will provide information essential to the development of accurate monitoring systems by testing the similarity and uniqueness of soil ecosystems across site gradients. These studies will also identify those soil species indicative of certain habitats or soil conditions which will provide more sensitive tools in the ongoing research of forest soil management. The research will allow for a more complete biodiversity inventory and taxonomy of soil biota in the northern interior, and will help lay the groundwork for further ecological studies of soil biota and site productivity.

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

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 nonmycorrhizal root proliferation (Berch et al. 2006), and shifts in forest dynamics to favour 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* x *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 (54º49'N 127º10'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 (54º40' to 47'N and 127º16' to 36'W) at approximately 900 m elevation. Mean annual air temperature of these sites is estimated, based on ClimateBC extrapolation (Spittlehouse 2006) at 2.3ºC, 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 (4 plant associations x 5 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 x 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 centre of each plot to determine stand basal area.

As described in Kranabetter et al. (2007), the mass per ha (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 persulphate solution, and forest floor and mineral soil N concentration data was converted to mass per ha 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 fifteen subalpine fir saplings and bulked together to form 3 subsamples per plot. Foliar samples were oven-dried (70°C for 24 hours), ground with a Wiley mill and analyzed for N by dry combustion.

Soil moisture was measured gravimetrically every three 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 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 x 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 3 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 x 200 root tips per sapling).

Each root tip was examined stereoscopically (10x to 40x magnification) for features such as colour, 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 1000X 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 1-3 tips were placed into a fast prep extraction tube containing AP1 solution of the DNeasy 96 Plant Kit (Qiagen, Mississauga CAN). 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 seconds,

then stored at -20°C for 10 minutes followed by centrifuging at 4000 rpm for 10 minutes. Six hundred μl of the AP3/E solution was added to 400 μl supernatant and the resultant solution was shaken vigorously for 15 seconds, centrifuged to 3000 rpm and then immediately stopped. One ml of each sample was added and vacuumed from each well of a new DNeasy plate. Four hundred μl 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 one minute and then vacuuming, after which this step was repeated. The DNeasy plate was centrifuged for 3 minutes at 2000 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 3130x1 capillary sequencer (Applied Biosystems, Foster City USA). 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 (5 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 (GLM) 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 x 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 nonmetric multidimensional 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 1). 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), 4 species of *Piloderma*, 7 of *Tomentella* and *Pseudotomentella*, 8 of *Russula*, 27 of *Cortinarius*, 2 of *Lactarius*, 1 of *Tricholoma*, and a variety of unknown fungi (Appendix 1; 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 1 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 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 – Devils club sites (Table 3), equal to an approx. 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 with soil N indices and foliar $N_{\%}$ (Table 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 6 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 favour 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 7 contact, 11 short-distance, 14 medium-fringe, 2 medium-mat, and 3 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 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 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 (Mittelbach 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 heterogenous 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 (Mittelbach 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 coloured *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 nonvascular 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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Table 1. Stand characteristics and understory *A. lasiocarpa* foliar N concentrations by plant association (means with SE in brackets).

Plant association*	n	Stand ht. (m)	Site index (m @ 50 yr)	Foliar $N_{\%}$ ($g\ kg^{-1}$)	Basal area ($m^2\ ha^{-1}$)	Relative cover (%) 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)

* soil nutrient regimes 'P' poor, 'M' medium, 'R' rich, 'VR' very rich

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

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*	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)

* soil nutrient regimes 'P' poor, 'M' medium, 'R' rich, 'VR' very rich

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

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 – Huck.berry (n = 5)	Rich – Oak fern (n = 5)	V. Rich – Devil's cl. (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 ($p < 0.05$).

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

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; $p < 0.001$; $r^2 = 0.71$

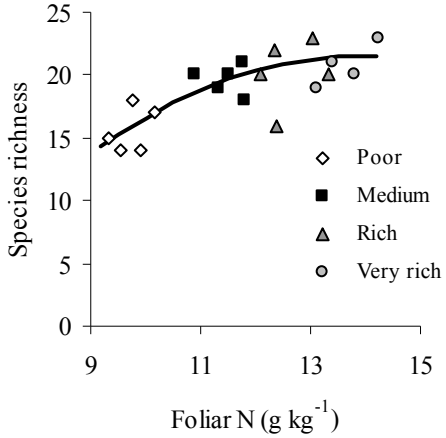


Fig. 2. Nonmetric multidimensional scaling analysis of EMF communities among the 19 plots (based on the abundance of 74 species), rotated to maximize correlation with inorganic N mass on axis 1.

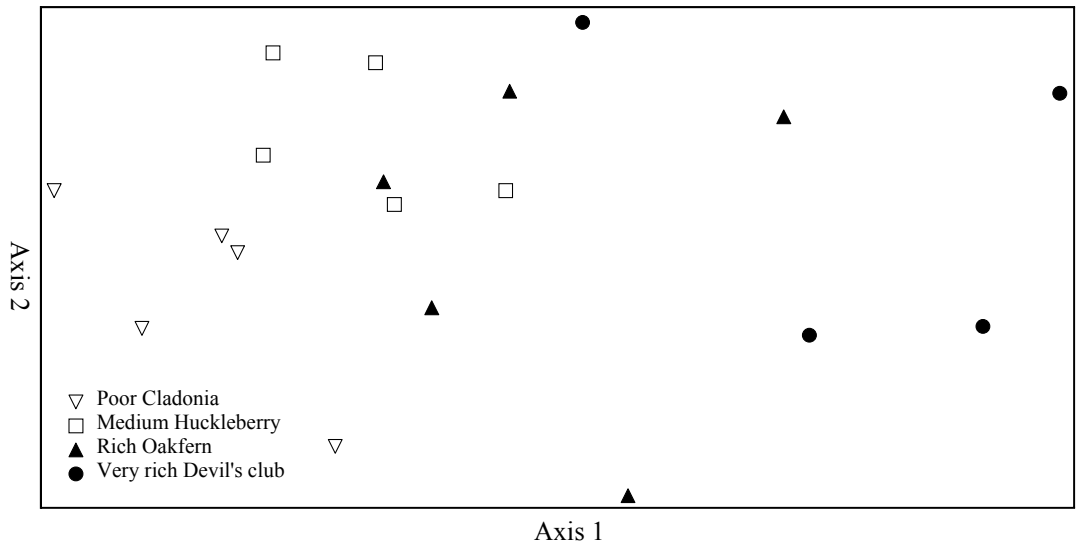


Fig. 3. Abundance of 6 EMF species in correlation with foliar N concentration of *A. lasiocarpa*.

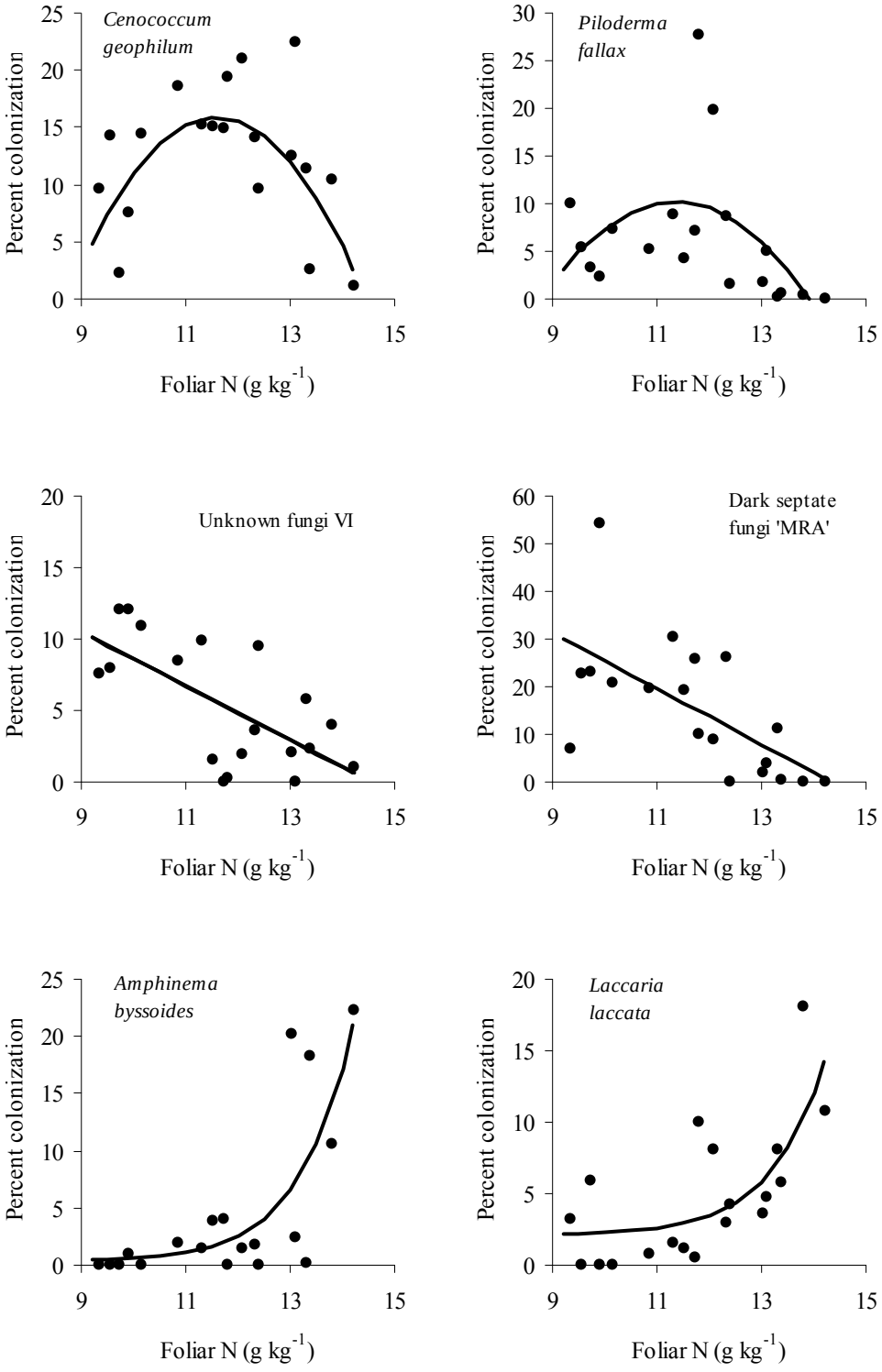
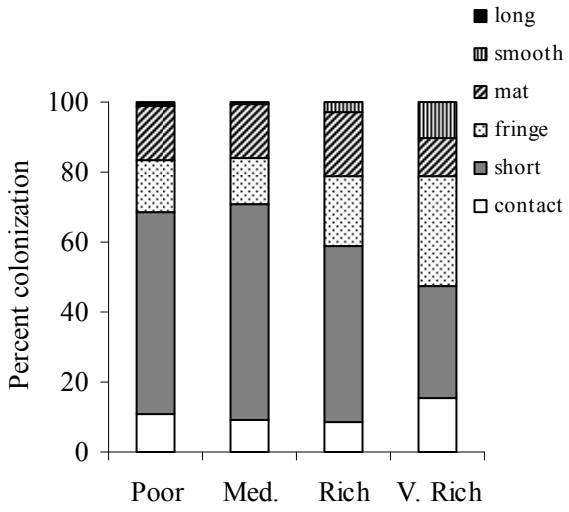


Fig. 4. Hyphal exploration types of EMF as a mean percent of root colonization grouped by plant association (n = 5 for poor, medium and rich plant associations, 4 for very rich).



Appendix 1. List of morphotypes with successful ITS rDNA analysis. 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.

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 colour 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.

3. Epigeous fruiting bodies of ectomycorrhizal fungi as indicators of soil fertility and associated nitrogen status of boreal forests

Introduction

Indicator species of migratory songbirds, raptors, small mammals, soil arthropods, epiphytic lichens and wood-decay fungi have been touted as useful fine-filter tools in ecosystem monitoring, especially in evaluating the retention of old-growth forest conditions and maintenance of biodiversity (Nilsson et al. 2001; Kremsater et al. 2003). Some of the criteria to consider in selecting indicator species are that they be readily quantifiable, that they represent key habitat features or processes, and that they be sensitive to ecosystem manipulation (Ferris and Humphrey 1999). Many soil biota, with the exception of select arthropods, have yet to be thoroughly assessed for suitability as indicators (Thompson 2006). Ectomycorrhizal fungi (EMF) deserve further study in this regard given the typically high species diversity of this guild and key roles these symbiotic fungi have in ecosystem function (Read et al. 2004). With taxonomic training, epigeous EMF fruiting bodies can be assessed efficiently over large areas and have proven useful in evaluating, for example, partial-cutting effectiveness (Luoma et al. 2004) and late-seral dependent EMF species (Kranabetter et al. 2005).

Besides monitoring old-growth forest habitat, indicator species of soil quality and productivity, especially as related to nitrogen (N), could be valuable in a number of applications (Knoepp et al. 2000). The sensitivity of EMF fruiting bodies to edaphic conditions is apparent from surveys of modified sites (e.g., Rűhling and Tyler 1990) and consequently the response of macrofungi to elevated N availability has received much attention. Evidence from primarily short-term, experimental research with N fertilizer applications suggest immediate declines in EMF sporocarp production and species richness with increased N availability, but with some positive responses in abundance for a subset of nitrophilic species (Brandrud 1995; Jonsson et al. 2000; Peter et al. 2001a; Avis et al. 2003; Edwards et al. 2004). Parallel studies from undisturbed forests with naturally contrasting levels of N availability are rare; Trudell and Edmonds (2004) reported lower sporocarp biomass in more productive forests, but more significant differences in species composition rather than richness with soil fertility. Natural and experimental N gradients are not entirely analogous, however, since N availability in

pristine forests is not an independent variable, but rather a property of the underlying soil moisture and geochemical drivers (Giesler et al. 1998). Conclusions on N effects should therefore be considered in an ecological context, and studies of undisturbed forest ecosystems would be helpful in fully exploring soil abiotic – biotic relationships (Erland and Taylor 2002). While there has been considerable effort in linking EMF macrofungi composition with site conditions (see review in Trudell and Edmonds 2004), confounding changes in host tree species or macroclimate with different forest types has make edaphic effects difficult to isolate.

In this study we report on the epigeous EMF macrofungi associated with replicated productivity gradients of upland southern boreal forests in British Columbia (Canada). The plots were situated in close proximity and elevation, with old-growth stands of *Pinus contorta* (Dougl. ex Loud), *Abies lasiocarpa* ([Hook.] Nutt.), and *Picea glauca* x *englemanni* ([Moench] Voss) at almost every site that created an ideal study design to minimize the effects of climate (O'Dell et al. 1999), stand age (Kranabetter et al. 2005), and host specificity (Durall et al. 2006) on relationships between soil fertility and EMF species distribution. A previous belowground EMF assessment with *A. lasiocarpa* documented strong associations of EMF species with soil conditions, in particular the amount and forms of N (organic N, NH_4^+ and NO_3^-), and an asymptotic correlation of species diversity with foliar N concentrations (Kranabetter et al. 2009). Some of the most prevalent EMF species on root tips from these sites (e.g., *Cenococcum geophilum*, *Piloderma* spp., *Amphinema byssoides*, *Tomentella* spp.) will not be represented aboveground, but macrofungi should provide more comprehensive distribution data for the many infrequent EMF species of forests (Taylor 2002). Ideally epigeous fruiting bodies will provide an effective indicator of ecosystem status, based on a subset of readily quantifiable EMF species (albeit over several years of monitoring), in addition to a more complete portrayal of the EMF community (Richard et al. 2005; Smith et al. 2007).

The objectives in the second phase of this productivity gradient analysis were to: 1) examine how closely EMF macrofungi assemblages and richness align with the plant associations and corresponding soil properties; 2) document the extent of widely distributed (multi-site) species versus more narrowly-distributed, specialist species which

could indicate N status of forest soils; and 3) provide EMF macrofungi community data from these largely pristine, old-growth forest ecosystems to help define benchmarks for ecological integrity (species composition, diversity, and functional organization) in future managed landscapes.

Materials and Methods

Site descriptions and study design

Study sites described previously (Kranabetter et al. 2009) were used for the macrofungal surveys, but with four replicates of each plant association (rather than five) for a total of 16 plots. Briefly, the plots were located at approximately 900 m elevation in the moist cold (mc) subzone of the Sub Boreal Spruce zone (SBS) near Smithers, British Columbia, Canada. Four plant associations (poor-Cladonia, medium-Huckleberry, rich-Oak fern, and very rich-Devil's club) from 5 blocks located within a 25-km area (54º40' to 47'N and 127º16' to 36'W) were sampled to provide a wide range in upland edaphic conditions. Each plot was 50 m x 30 m (0.15 ha) in size.

Almost all plots had mixed, old-growth coniferous forests (~ 180 years), but with differences in relative canopy composition across the gradient (Table 1). In addition to the co-dominant canopy, a very minor amount of *Tsuga heterophylla* (Raf. Sarg.) was found regenerating in the understory of four plots. Possible EMF hosts among shrub species (Hagerman et al. 2001) included *Alnus viridis* ([Chaix.] D.C.), *Arctostaphylos uva-ursi* ([L.] Spreng.), *Amelanchier alnifolia* ([Nutt.] Nutt.), and *Shepherdia canadensis* ([L.] Nutt.). A contribution of sporocarps by understory western hemlock and shrub species is perhaps possible but we expect this is to be a very minor influence compared to the dominant conifer species of the forest canopy.

Foliar N concentrations ($N_{\%}$) of the regenerating *Abies lasiocarpa* understory and selected soil properties (kg ha^{-1}, upper mineral soil and forest floor combined, with the exception of pH) were tested against EMF macrofungi communities, including a 5 week in situ incubation of dissolved inorganic and organic N (DIN + DON), average gravimetric moisture from May to September, organic P, total S, exchangeable cations (Ca, Mg, and K) and pH of mineral soil and forest floor (N indices and soil moisture listed in Table 1). Methodologies for quantifying these properties, along with further

descriptions of stand, soil and vegetation characteristics of the study plots, are listed in Kranabetter et al. (2007).

Fruiting body surveys

Data on epigeous EMF macrofungi species were collected over a three year period, from 2006 to 2008. The plots were checked twice each fall during the expected peak in fruiting (August 20-23 and September 11-14) for a total of 6 sample periods. A species list was generated by searching the entire plot (0.15 ha) during each of the sample periods. Total species richness per site was determined from the cumulative species list collected over the 3 years. Species abundance was quantified by five 15 m transect lines, measuring 1 m in width, randomly located perpendicular to the central axis of the plot. A species found on 2 of the 5 transects within a plot, for example, would have an abundance value of 40%. A species found within the plot, but not on any transects, was given an abundance value of 5%. The locations of the transects were randomly reassigned (without replacement) for each of the 6 sample periods. The values from the 6 sample periods (including 0 if absent) were used to determine an average abundance for the comparison of EMF communities.

Taxonomic identification followed Thiers (1982), Moser (1983), Breitenbach and Kranzlin (2000), Bessette et al. (2000), Brandrud et al. (1990), and Tylutki (1987). In addition to the identified species, there were a few *Cortinarius* taxa, especially small, brown *Telamonia* (e.g., section *Armeniaci*), which were too difficult to consistently identify to species and were consequently underrepresented in these survey results. Taxa with numerous subspecies, such as *C. brunneus* and *C. flexipes*, were not separated further in our surveys and identified collectively as a species group. We did not sample hypogeous fungi because of the soil disturbance required to find these fruiting bodies. Representative voucher specimens were dried and deposited at the University of British Columbia herbarium. A subset of species underwent ITS rDNA analysis using the methodology described in Kranabetter et al. (2009) for accession into GenBank (Appendix 1). Voucher photographs of forty *Cortinarius* species were also taken to support our taxonomic identification and will be available through MatchMaker (British Columbia Ectomycorrhizal Research Network 2007).

Statistics

The four replicates of each plant association over five blocks represents a balanced incomplete block design that meets the requirement for connectedness to undergo statistical analysis (Mead 1988). Mean species richness by plant association was determined under Proc Mixed using the Estimate function (SAS 2004), with significant differences in species richness tested in pairwise comparisons. The general linear model procedure in SAS using Type 1 Sums of Squares was used to test linear and curvilinear (polynomial, exponential, logarithmic and power) correlations between plot means of dependent and independent variables (n = 16). Goodness of fit was evaluated using r^2 and step-wise elimination of variables. No significant effect of block or block x treatment interactions was found in any of the correlations.

A comparison of EMF macrofungal communities among plots was undertaken by non-metric multidimensional scaling (NMS), using the Sorenson (Bray-Curtis) distance measure for species presence/absence and relative Sorenson 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. EMF community similarity between plant associations based on species presence/absence was determined with the Jaccard similarity index (Mueller-Dombois and Ellenberg 1974).

EMF species were classified into distribution categories based on the extent of fruiting body frequency and abundance along the productivity gradient as follows: "broadly tolerant" species had a minimum 50% frequency in fruiting for each of the four plant associations; "partially intolerant" species of poorer or richer soils had a minimum 50% frequency in three of the four plant associations, or three of the four plant associations where sporocarp abundance was < 2%; partially intolerant species of soil extremes had 50% frequency in the medium and rich plant associations, with less frequent or < 1% sporocarp abundance in either poor or very rich plant associations; and "specialist" species had 75% frequency in no more than two plant associations, or 50% in either plant association where abundance > 1%. The remaining species, termed "satellites" (Gibson et al. 1999), were too infrequent to meet any of the above criteria for classification.

Results

We identified 176 epigeous EMF species from 19 genera over the 3 year survey period. The most species-rich genus was *Cortinarius* (73 species), followed by *Russula* (25), *Tricholoma* (15), *Hygrophorus* (13), *Lactarius* (10), *Inocybe* (8), *Suillus* (4), and three or fewer species for the remaining genera (Appendix 1). Fruiting varied almost two-fold between sample years, peaking in 2007 with a total of 168 species across all plots, followed by 2008 (137 species) and 2006 (85 species).

Cumulative species richness for the 3 year period ranged from 39 to 89 species per plot (0.15 ha), and was consistently lowest on poor-Cladonia sites ($p < 0.001$), averaging 48 species, compared to 75 for medium-Huckleberry, 80.5 for rich-Oak fern, and 70.5 for very rich-Devil's club sites (the difference in species richness between the latter two plant associations was also significant [$p = 0.048$]). Likewise, species richness followed a skewed parabolic correlation with soil productivity, as expressed either by foliar N concentration of *A. lasiocarpa* or N availability of the soil profile (Fig. 1). Positive linear correlations in species richness by genera were found for *Inocybe*, *Russula* and *Lactarius* along the N gradient, while *Cortinarius*, *Tricholoma* and *Hygrophorus* had curvilinear correlations in species richness with foliar $N_{\%}$ (Fig. 2).

The community NMS analysis of macrofungi (Fig. 3a, b) showed a progressive separation by plant association that closely followed the productivity rankings for both EMF species presence/absence ($r^2 = 0.904$, final stress of 11.2 after 29 iterations) and EMF species abundance ($r^2 = 0.910$, final stress of 10.8 after 25 iterations). The only exception was the first replicate of the very rich-Devil's club sites (Table 1), which was more closely aligned to the rich-Oak fern plots in both ordinations. Only one axis was generated by the NMS analysis because all of the ordinated distances between plots closely matched the rank-order of the original distance matrix. Pearson and Kendall correlations for EMF species abundance were most significant between axis 1 and soil N indices, including DIN + DON mass ($r^2 = 0.800$), inorganic N mass alone ($r^2 = 0.798$) and DIN:DON ratio ($r^2 = 0.709$).

A conceptual model of macrofungi distribution is proposed to illustrate the relative extent of habitat occupied by species of the EMF community (Table 2; see Appendix 1 for a complete species list by distribution class). The most widespread EMF species were found fruiting over 100% of the productivity gradient and so were

characterized as broadly tolerant to the complete range of soil properties of this upland landscape. Next were species that were slightly narrower in distribution, found over approx. 70% of the soil gradient, that were partially intolerant of the richest soils, the poorest soils, or the soil extremes. The most constricted distribution of species was termed the specialists, each of which occupied approx. 40% of the productivity gradient. These species were narrow enough in distribution along the productivity gradient, despite a degree of overlap, to collectively indicate oligotrophic, mesotrophic, and eutrophic soil nutrient regimes (Fig. 4). Lastly were the satellite species, which were too infrequent and low in abundance to characterize effectively into any of the distribution classes. Exceptions in the classification criteria for broadly tolerant were made for *R. gracilis* and *R. xerampelina*, both of which were more uncommon on medium sites than the gradient extremes.

Overall, the number of broadly tolerant, partially intolerant and specialist species was 110, comprising the core of the EMF community, with the remaining 66 as satellite species (equal to 14%, 26%, 23% and 37% of the total species, respectively). The mix of tolerant and partially intolerant species along the gradient resulted in a moderate degree of similarity (Jaccard index) between EMF communities, averaging 50% (range 29-69%) in pairwise comparisons between plant associations.

Discussion

Ectomycorrhizal macrofungal communities were remarkably consistent between replicates and strongly aligned with plant associations, in support of the belowground EMF assessment, and the results demonstrate again a very high degree of EMF community specialization in relation to soil properties within climax forests (Gehring et al. 1998; Toljander et al. 2006; Robertson et al. 2006). As expected, only a handful of macrofungal species from the surveys mirrored their belowground abundance to any degree (e.g., *L. laccata*, *C. semisanguineus*, *C. hemictrichus*, *R. decolorans*, *R. bicolor*), and the fruiting body data provided a disparate yet complementary view of EMF community composition and structure along soil fertility gradients (Kranabetter et al. 2009). We used the N status of soils and foliage to define oligotrophic, mesotrophic and eutrophic site conditions, but recognize that these N indices covary with a number of properties in these natural systems (Nordin et al. 2001; Kranabetter et al. 2007). Possible

influences on EMF species distribution beyond N regime could include, for example, tolerance to soil droughtiness or adaptations for P uptake. Nevertheless, the strong correlations between species assemblages and DIN + DON availability or DIN:DON ratios certainly support a fundamental link between EMF species distribution with organic N, NH_4^+ and NO_3^- dynamics (Lilleskov et al. 2001; Avis et al. 2003; Trudell and Edmonds 2004).

It was not possible to locate sites with an equal distribution of host tree species, and the lower host abundance (notably *Picea*) on some poor-Cladonia sites may have exaggerated the reduction in EMF species richness on these most stressful sites as compared to the decline measured belowground (40% reduction versus 25%, respectively; Kranabetter et al. 2009). Otherwise the differences in species richness among sites were relatively small (5 to 10 species or a 6 to 12% decline for medium-Huckleberry and very rich-Devil's club, respectively, compared to rich-Oak fern sites), and the shifts in EMF species composition along the N gradient were much more indicative of soil conditions than diversity measures. It was interesting to note in this regard that the very rich-Devil's club outlier from both ordinations was actually consistent with N regime because nitrification was not detected in this replicate (Table 1), which suggests the presence of some mesotrophic species in this plot was not entirely unusual.

The curvilinear trend in species richness did not match the decline in EMF species richness generally reported for artificial N gradients (Jonsson et al. 2000; Peter et al. 2001a; Avis et al. 2003), and this discrepancy may reflect in part the inordinately high concentrations of inorganic N created under experimental fertilizer treatments (Toljander et al. 2006). With emigration and immigration of EMF species over time it might be possible for a better adapted community to develop in response to the enhanced soil N status of a forest, but this would also depend upon the sensitivity of these EMF species to the other soil properties defining eutrophic habitat.

Trends by genera also indicated broad patterns in community response to N that might be useful in examining related forest landscapes (Lilleskov et al. 2001; Avis et al. 2003; Trudell and Edmonds 2004). Many *Inocybe*, *Lactarius* and *Russula* species favoured richer soils with elevated concentrations of inorganic N, whereas *Cortinarius*,

Tricholoma and *Hygrophorus* species richness peaked on mesotrophic sites with predominantly organic N cycles. Nevertheless, many species within genera occupied all manner of site types (Table 2), so detailed information on core species autecology would perhaps be more useful than simple comparisons among genera in understanding modified ecosystems. Macrofungi biomass was not measured in this study, but we noted how many species of oligotrophic soils produce large sporocarps (e.g., *R. decolorans*, *R. cascadensis*, *T. magnivelare*) in frequent shiros that would be consistent with the peak in biomass production reported elsewhere (Peter et al. 2001a; Trudell and Edmonds 2004). It should be emphasized that three years of surveys is likely to be the minimum required for these interpretations to accommodate the natural variation in macrofungal production (Krebs et al. 2008).

A diverse mix of both overlapping and exclusive species distributions along environmental gradients is challenging to synthesize (Whittaker 1975), and in the belowground assessment a simple distinction was drawn only between multi-site and narrowly distributed fungi. This concept was subjectively expanded upon using the more comprehensive frequency data of macrofungi, while recognizing that abundance was not necessarily uniform among species within each distribution category. In this way the diverse mix of habitats for the core EMF species could be well described by seven possible distribution classes; *R. xerampelina* was the only species to portray a possible 'U' shaped response to N availability, but this may be as likely due to chance in plot selection rather than a particularly unique distribution. An alternative to this intermediate focus would be individual response curves to the N gradient based on sporocarp abundance, but the interpretations would be debatable (especially for host-specific EMF species) given the variable proportions in tree species cover. At the very least, these distribution categories provide possible hypotheses for evaluating niches and specialized functions of core EMF species (Nygren et al. 2007; Cajsa et al. 2008).

The large number of infrequent and possibly transient satellite species is a common feature of EMF communities (Smith et al. 2002; Richard et al. 2004). The role of satellite species is perhaps not immediately measureable by ecosystem production, but plant ecologists suggest these rarer species likely provide future recruitment potential into the core community (Grime 1998; Gibson et al. 1999). Thus in the long term these

satellite EMF species might play an intermittent yet important role in providing a species reservoir for ingress in response to ongoing pedogenic processes (podzolization, nutrient sequestration) or possible changes in climatic regime and host species distribution.

A specialization in EMF communities by site type would arguably provide some of the adaptations required for relatively few tree species to occupy vast, complex landscapes. Diverse ectomycorrhizal fungal communities with mixed distribution classes likely support this ecosystem productivity and resiliency (Perry and Amaranthus 1997), but under widespread stressors some fragmentation of these communities may occur, as indicated perhaps by a greater prevalence of broadly tolerant, multi-seral EMF species at the expense of specialist, late-seral species. Changes in EMF communities of this nature could represent a loss in ecosystem integrity, which can be briefly defined here as the capacity of an ecosystem to support and maintain a balanced, integrated, adaptive community of organisms similar to undisturbed ecosystems of a region (Frego 2007). The focus in ecosystem monitoring could include attributes related to functional organization, such as the total number of EMF species by genera, the proportion of species per distribution class, and the ratio of satellite to core species. Given the challenges in sampling infrequent EMF species belowground, we would suggest there are unlikely to be any simple surrogates of ecosystem integrity related to EMF communities that does not involve surveys of fruiting bodies.

Conclusions

Similar to plants as bioindicators (Klinka et al. 1989), the potential of epigeous EMF fruiting bodies to provide sensitive indicators of soil fertility was well supported by the approx. 40 specialist species that were collectively aligned by simply presence or absence to the N status of these boreal ecosystems. Corresponding indicator species for related landscapes would require site-specific community data, however, since EMF species composition would undoubtedly vary a considerable degree across biomes (Dahlberg et al. 1997; Peter et al. 2001b). Where appropriate (e.g., common host species, uniform stand ages), a monitoring program of EMF fruiting bodies could be a relatively inexpensive, nondestructive, and thorough indication of functional changes in soil fertility and associated N cycles that might arise under anthropogenic activity. In addition, epigeous EMF species richness and the distribution of tolerant, specialist and

satellite species could provide useful indicators of functional organization for evaluating ecosystem integrity. The EMF macrofungi communities of late-seral forests under current environmental conditions should therefore become especially important reference benchmarks given the possible widespread altered forest landscapes of the future (Hamann and Wang 2006).

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Table 1. Selected stand and soil properties of the plots surveyed for epigeous EMF fruiting bodies.

Plant association	Age (yrs)	Stand ht. (m)	Pl (%)	Bl (%)	Sx (%)	Moisture (kg ha^{-1})	DON (kg ha^{-1})	NH_4^+ (kg ha^{-1})	NO_3^- (kg ha^{-1})	DIN: DON ratio
P – Cladonia	180	20.2	80	20	0	16	26	0.3	0	1
P – Cladonia	190	16.8	83	17	0	13	18	1.5	0	8
P – Cladonia	115	23.1	79	11	11	11	14	0.9	0	6
P – Cladonia	165	24.9	88	3	9	17	15	1.3	0	9
M - Huckleberry	185	27.3	57	34	9	17	28	1.8	0	6
M - Huckleberry	180	26.5	36	62	2	14	22	1.9	0	9
M - Huckleberry	173	27.3	51	41	8	21	32	4.3	0	14
M - Huckleberry	205	29.1	32	58	10	27	25	6.5	0	26
R – Oak fern	180	31.4	25	67	8	23	28	5.6	0	20
R – Oak fern	179	32.1	5	90	5	21	36	5.9	0	17
R – Oak fern	188	31.5	26	36	38	40	34	11.2	0.3	34
R – Oak fern	177	30.5	7	79	14	33	35	7.1	0.1	21
VR - Devil's Club	174	35.3	23	64	13	28	34	4.5	0	13
VR - Devil's Club	185	34.6	22	62	16	25	28	8.1	15.1	83
VR - Devil's Club	178	37.8	19	64	17	32	26	11.2	3.8	59
VR - Devil's Club	206	35.9	12	77	12	26	40	13.1	3.1	40

Notes: Soil nutrient regimes 'P' poor, 'M' medium, 'R' rich, 'VR' very rich. Canopy composition % cover estimated visually and includes co-dominant and subdominant canopies. 'Pl' = lodgepole pine, 'Bl' = subalpine fir and 'Sx' = hybrid white spruce. 'DIN' = dissolved inorganic N, 'DON' = dissolved organic N.

Table 2. Conceptual depiction of core EMF species distribution along fertility gradients, as expressed by *A. lasiocarpa* foliar N concentration and soil N availability. Three EMF species are given as examples under each category, with the total number of species per distribution class in brackets, and the extent of the distribution along the productivity gradient indicated by the dashed lines.

		Foliar N (g kg^{-1})		
9.5	10.8	12.0	13.3	14.5
		DIN + DON (kg ha^{-1})		
10	22.5	35	47.5	60

<---------- **Broadly tolerant** (24) ---------->

Cortinarius cinnamomeus

Laccaria laccata

Lactarius rufus

Intolerant of richest soils (12) ---------->

Cortinarius gentilis

Cortinarius semisanguineus

Leccinum aurantiacum

<---------- **Intolerant of poorest soils** (23)

Cortinarius hemictricus

Cortinarius sodagnitus

Russula bicolor

<---------- **Intolerant of soil extremes** (10) ---------->

Cortinarius renidens

Lactarius kaufmanii

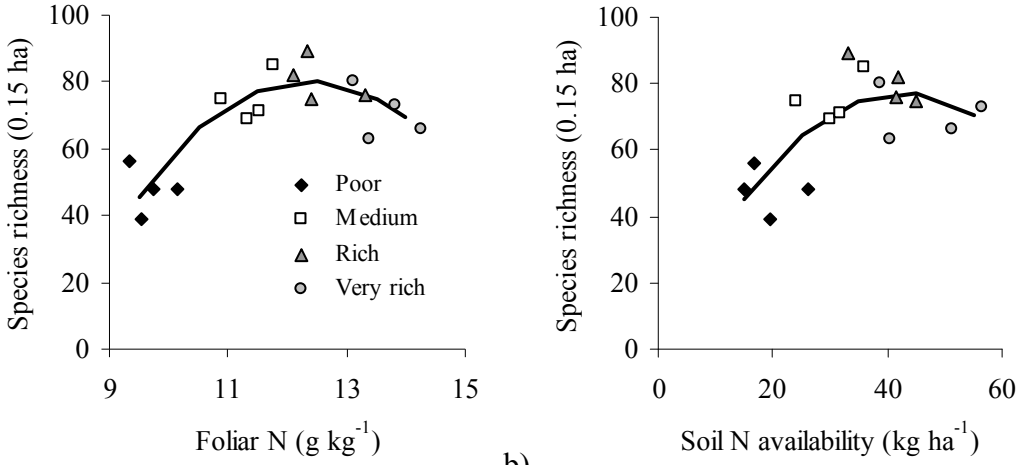
Rozites caperata

<------ **Oligotrophic** (13) ---->	<----- **Mesotrophic** (10) ----->	<-------- **Eutrophic** (18) ----->
Boletopsis subsquamosa	*Cortinarius alboviolaceus*	*Cortinarius elegantior*
Cortinarius pinophilous	*Hygrophorus saxatilis*	*Inocybe geophylla*
Tricholoma magnivelare	*Russula occidentalis*	*Russula cessans*

Fig. 1. EMF species richness in correlation with a) *A. lasiocarpa* foliar N concentrations (g kg^{-1}) and b) soil N availability (DIN+DON mass) (kg ha^{-1}).

Species richness (0.15 ha) = -540 + 99.9($N_{\%}$) – 4.0($N_{\%}$)^2; $p < 0.001$; $r^2 = 0.73$

Species richness (0.15 ha) = -0.1 + 3.7(Soil N) – 0.04(Soil N)^2; $p = 0.007$; $r^2 = 0.53$



a) b)

Fig. 2. Species richness per plot (0.15 ha) by genera in correlation with foliar N concentrations (g kg^{-1}) of *A. lasiocarpa*.

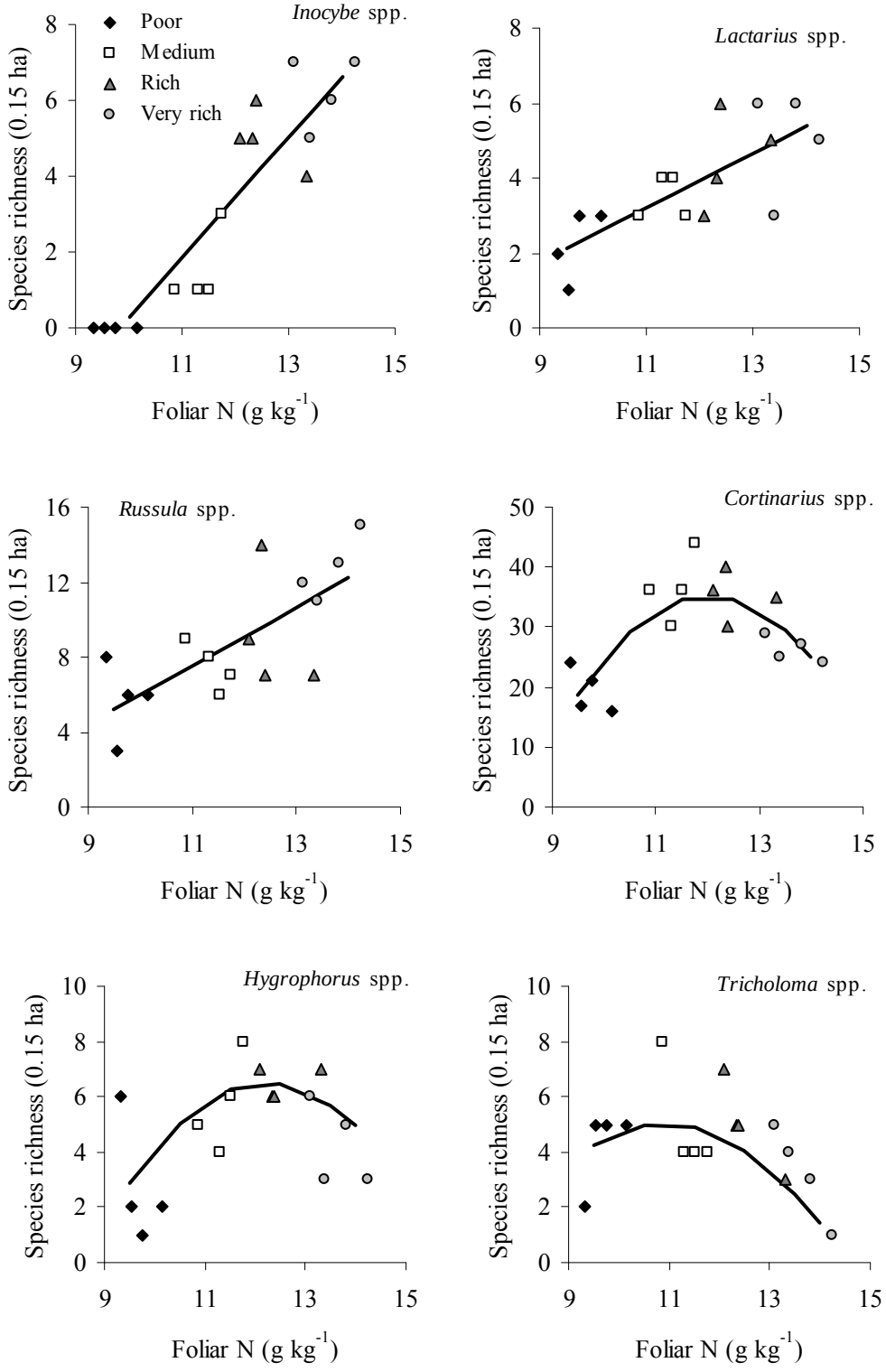
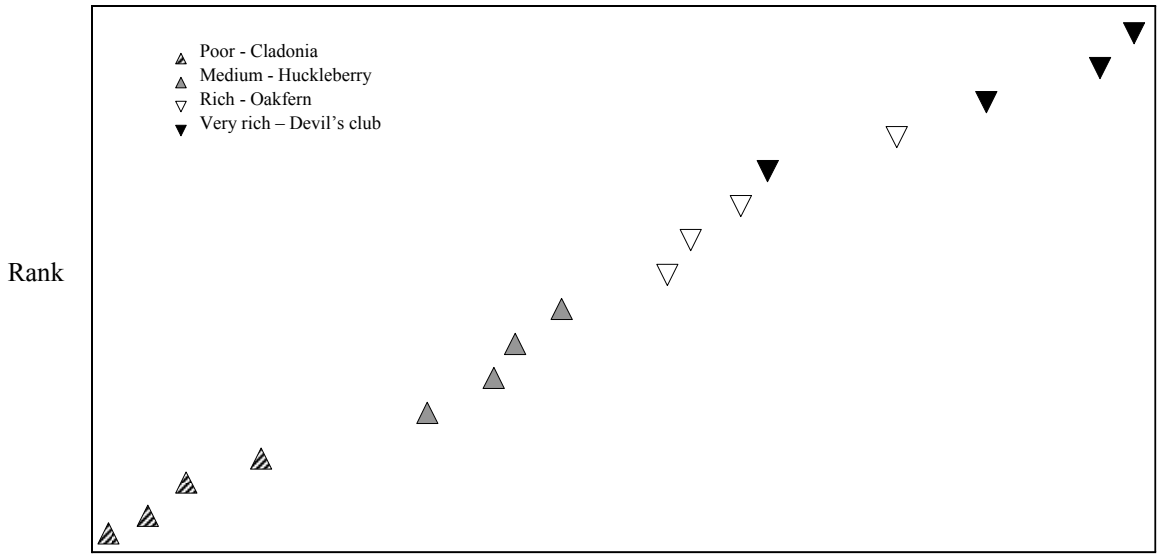


Fig. 3. Non-metric multidimensional scaling ordination of EMF sporocarp communities (176 species) among plant associations (poor-Cladonia, medium-huckleberry, rich-Oak fern, and very rich-Devil's club) based on a) species presence/absence and b) species abundance (n = 16).

a)



b)

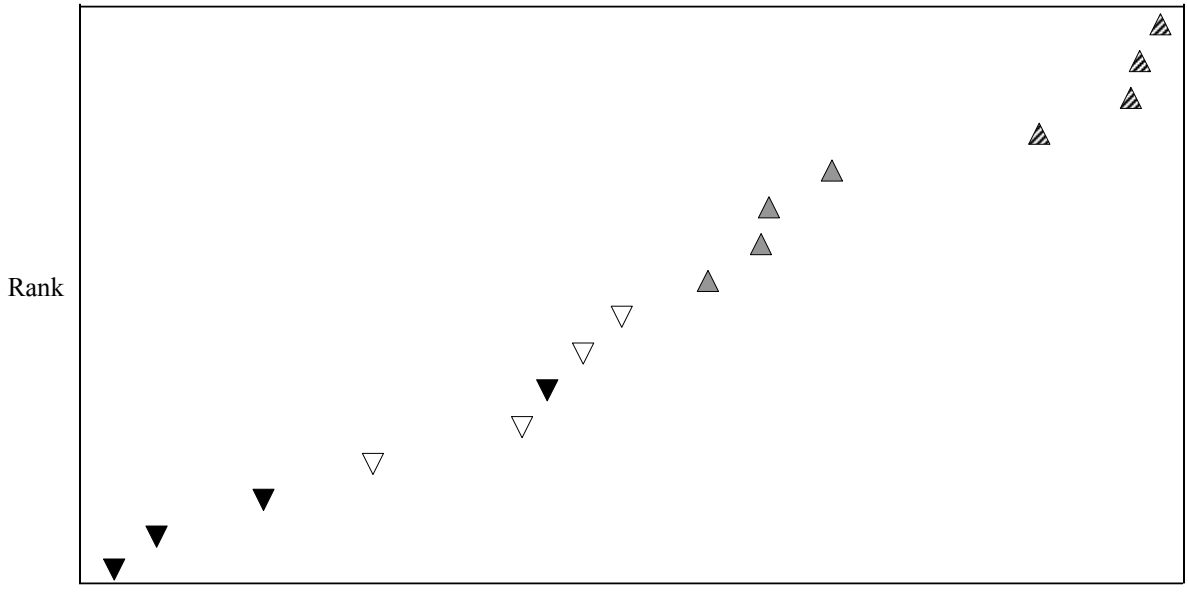
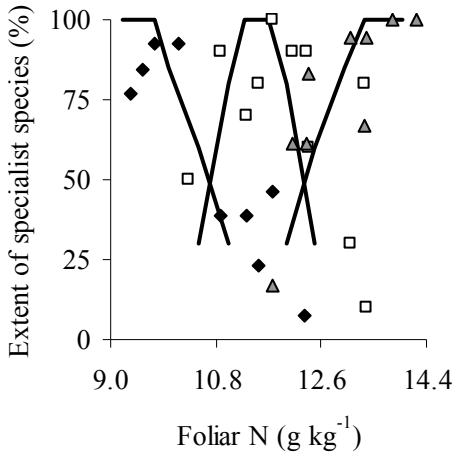


Fig. 4. The extent of specialist EMF species found per plot as a percent of the total for each nutrient regime (oligotrophic [13 species] = black diamonds; mesotrophic [10]= open squares; eutrophic [18] = gray triangles) in relation to *A. lasiocarpa* foliar N concentrations. Distribution curves idealized to show nutrient regimes covering 40% of the foliar N gradient.



Appendix 1. Mean abundance (% frequency in brackets) of EMF species among the four plant associations. Species are listed alphabetically within distribution categories of broadly tolerant, partially intolerant (poor, rich and extremes), specialist (oligotrophic, mesotrophic and eutrophic) and satellite species.

Ectomycorrhizal fungal species	Poor – Cladonia (n = 4)	Medium – Huck.berry (n = 4)	Rich – Oak fern (n = 4)	V. Rich – Devil's cl. (n = 4)	GenBank accession #
Broadly tolerant					
Chroogomphus vinicolor	2.7 (100)	5.4 (100)	6.3 (100)	4.0 (50)	
Cortinarius acutus	0.4 (50)	2.9 (75)	4.0 (100)	2.9 (100)	FJ039609
Cortinarius brunneus grp	2.5 (100)	4.6 (100)	3.3 (100)	1.9 (100)	FJ039682
Cortinarius cinnamomeus	1.3 (75)	12.9 (100)	9.2 (100)	2.9 (100)	
Cortinarius croceus	2.1 (75)	10.2 (100)	1.3 (75)	1.0 (75)	
Cortinarius flexipes grp	0.6 (75)	2.9 (75)	4.2 (100)	5.8 (100)	
Cortinarius laniger	1.3 (75)	2.3 (100)	4.8 (100)	1.0 (75)	FJ039557
Cortinarius obtusus	1.5 (100)	3.5 (100)	6.3 (100)	0.6 (50)	FJ039610
Cortinarius paragaudis	0.4 (50)	3.5 (100)	4.8 (100)	1.3 (100)	FJ039675
Cortinarius triformis	1.0 (50)	1.0 (100)	0.4 (50)	0.4 (50)	FJ039573
Cortinarius vibratilis	2.3 (100)	6.0 (100)	3.1 (75)	2.9 (100)	
Hebeloma crustiliniforme	0.4 (50)	2.1 (100)	8.8 (100)	4.2 (100)	
Hydnum repandum	0.6 (50)	0.6 (50)	2.3 (100)	0.6 (50)	
Laccaria bicolor	0.4 (50)	2.7 (75)	1.7 (75)	0.8 (75)	
Laccaria laccata	2.3 (75)	11.3 (100)	6.3 (100)	3.5 (100)	
Lactarius deliciosus	0.6 (50)	2.9 (75)	3.3 (100)	1.7 (100)	
Lactarius rufus	11.5 (100)	17.5 (100)	5.8 (75)	2.3 (75)	
Russula gracilis	0.4 (50)	0.2 (25)	1.0 (100)	3.5 (100)	
Russula cf *vinosa*	1.3 (50)	3.1 (100)	2.5 (75)	0.4 (50)	
Russula xerampelina grp	1.0 (75)	-	0.8 (50)	1.7 (75)	
Suillus brevipes	2.3 (100)	1.9 (75)	2.1 (75)	1.5 (100)	
Suillus flavidus	9.4 (100)	14.2 (100)	12.9 (75)	4.4 (50)	
Suillus tomentosus	21.3 (100)	12.5 (100)	2.7 (100)	1.0 (50)	
Tricholoma saponaceum	1.3 (100)	3.1 (100)	3.5 (75)	1.7 (50)	

Intolerant of richest soils					
Cortinarius boulderensis	0.6 (75)	2.3 (100)	0.6 (50)	0.2 (25)	FJ039558
Cortinarius cacoacolor	1.3 (75)	1.0 (75)	0.6 (75)	0.2 (25)	FJ03967
Cortinarius clandestinus	3.8 (100)	3.8 (100)	5.2 (100)	0.4 (25)	FJ039583
Cortinarius gentilis	14.2 (100)	3.3 (100)	0.8 (75)	-	FJ039686
Cortinarius muscigenus	2.9 (100)	2.1 (100)	1.5 (75)	0.2 (25)	FJ039578
Cortinarius niveoglobosus	0.4 (50)	0.4 (50)	0.2 (25)	-	FJ039676
Cortinarius phoenicius	2.3 (100)	1.3 (75)	0.6 (75)	0.2 (25)	FJ039599
Cortinarius pluvius	0.4 (50)	0.4 (50)	0.2 (25)	-	
Cortinarius semisanguineus	17.1 (100)	10.8 (100)	2.5 (75)	-	FJ039598
Hydnellum peckii	6.0 (100)	1.9 (75)	0.4 (50)	-	
Hygrophorus hypothejus	7.5 (100)	5.4 (100)	0.4 (50)	-	
Leccinum aurantiacum	2.9 (100)	1.7 (100)	0.8 (50)	-	
Intolerant of poorest soils					
Clavariadelphous pistillaris	-	2.7 (100)	4.0 (100)	2.9 (100)	
Cortinarius aureofulvous	-	0.6 (50)	0.6 (75)	0.6 (50)	FJ039644
Cortinarius argutus	-	0.2 (25)	1.5 (75)	0.8 (75)	FJ039662
Cortinarius calochrous grp	-	0.6 (50)	6.0 (100)	1.3 (75)	FJ039639
Cortinarius calopus	-	4.4 (75)	3.8 (100)	1.9 (75)	FJ039572
Cortinarius cana-barba	-	0.4 (50)	1.7 (100)	0.8 (75)	FJ039562
Cortinarius evernius	-	1.3 (75)	1.0 (75)	0.8 (75)	FJ039684
Cortinarius cf *flos-paludis*	0.2 (25)	3.3 (75)	2.3 (100)	2.7 (100)	FJ039560
Cortinarius hemictricus	-	3.5 (100)	6.0 (100)	5.0 (100)	FJ039543
Cortinarius sodagnitus	-	0.6 (50)	3.8 (100)	2.9 (100)	FJ039638
Cortinarius spilomeus	0.2 (25)	0.8 (75)	5.4 (100)	4.4 (100)	FJ039659
Cortinarius cf *pini*	-	0.2 (25)	0.6 (75)	0.4 (50)	FJ039614
Hebeloma mesophaeum	-	1.0 (75)	3.5 (100)	3.1 (100)	
Hygrophorus pudorinus	-	0.4 (50)	1.9 (50)	1.0 (50)	
Inocybe lanuginosa	-	1.3 (50)	1.5 (50)	1.3 (50)	
Inocybe pseudoastospora	-	0.4 (50)	1.5 (50)	1.0 (50)	
Ramaria rasilispora	-	0.2 (25)	0.2 (25)	1.3 (75)	
Russula aeruginea	-	0.2 (25)	0.2 (25)	1.9 (100)	
Russula bicolor	-	10.0 (100)	18.3 (100)	6.3 (100)	

Russula sanguinea	-	0.4 (25)	0.4 (25)	1.5 (50)	
Russula silvicola	-	0.8 (75)	0.8 (75)	1.0 (100)	
Tricholoma platyphyllum	-	2.7 (100)	2.9 (100)	1.0 (75)	
Tricholoma vaccinum	-	0.6 (75)	1.9 (50)	0.4 (50)	
Intolerant of extremes					
Chroogomphus ochraceus	1.0 (50)	3.5 (75)	2.5 (75)	0.4 (25)	
Cortinarius anomalus	0.2 (25)	1.3 (75)	1.3 (50)	0.8 (75)	FJ039605
Cortinarius biformis	0.2 (25)	0.6 (50)	0.6 (75)	0.6 (50)	FJ039574
Cortinarius renidens	0.2 (25)	1.9 (100)	5.2 (100)	0.8 (50)	FJ039665
Hygrophorus camarophylus	0.2 (25)	0.6 (75)	0.2 (25)	0.2 (25)	
Hygrophorus piceae	0.4 (25)	9.0 (100)	4.4 (100)	0.8 (25)	
Lactarius caespitosus	-	2.7 (75)	2.3 (100)	0.8 (75)	
Lactarius kaufmanii	0.4 (25)	6.0 (100)	8.8 (100)	0.8 (50)	
Rozites caperata	0.2 (25)	6.7 (100)	4.2 (75)	0.8 (75)	
Sarcodon imbricatus	0.4 (25)	0.6 (50)	2.7 (50)	0.4 (25)	
Oligotrophic					
Boletopsis subsquamosa	1.3 (75)	-	-	-	
Cortinarius pinophilous	7.9 (100)	1.3 (100)	-	-	FJ157123
Cortinarius traganus	1.3 (50)	0.2 (25)	-	-	FJ039570
Hygrophorus purpurescens	1.7 (75)	0.2 (25)	0.2 (25)	-	
Phellodon tomentosus	6.5 (100)	0.8 (100)	-	-	
Russula cascadensis	9.6 (100)	0.2 (25)	-	-	
Russula decolorans	6.5 (75)	0.6 (50)	-	-	
Russula densifolia	5.2 (100)	-	-	-	
Sarcodon scabrosum	4.2 (100)	-	-	-	
Suillus glandulosipes	1.3 (100)	1.0 (25)	-	-	
Tricholoma focale	1.5 (100)	1.0 (25)	-	-	
Tricholoma magnivelare	0.6 (75)	-	-	-	
Tricholoma sejunctum	1.7 (75)	2.0 (100)	-	-	
Mesotrophic					
Cortinarius alboviolaceus	-	1.5 (100)	0.4 (50)	0.4 (25)	FJ039575

Cortinarius camphoratus	-	0.6 (25)	2.7 (50)	-	FJ039626
Cortinarius ochrophyllus	0.4 (25)	0.8 (75)	0.4 (50)	-	FJ039604
Cortinarius purpurascens	-	0.8 (75)	1.0 (100)	0.2 (25)	FJ039660
Cortinarius scaurus	-	0.6 (75)	0.4 (50)	-	FJ039621
Hygrophorus erubescens	0.2 (25)	0.4 (50)	1.7 (100)	-	
Hygrophorus saxatilis	0.2 (25)	5.6 (100)	2.7 (100)	-	
Russula occidentalis	0.2 (25)	2.9 (100)	0.4 (50)	-	
Russula raoultii	-	0.6 (50)	1.5 (75)	0.6 (25)	
Tricholoma atrosquamosum	-	0.6 (50)	2.1 (75)	0.2 (25)	
Eutrophic					
Clavariadelphous truncatus	-	-	1.7 (75)	1.3 (75)	
Cortinarius elegantior	-	-	3.1 (100)	2.9 (100)	
Cortinarius guttatus	-	-	0.6 (75)	0.4 (50)	FJ039646
Cortinarius venetus	-	-	1.3 (75)	1.5 (50)	FJ039688
Hebeloma saarchiolens	-	-	0.8 (50)	0.8 (100)	
Hygrophorus chrysodon	-	-	0.2 (25)	0.8 (75)	
Hygrophorus olivaceoalbus	-	-	0.2 (25)	2.3 (75)	
Hygrophorus pustulatus	-	0.2 (25)	5.2 (75)	1.9 (100)	
Inocybe cf *friesii*	-	-	2.3 (50)	10.2 (100)	
Inocybe geophylla	-	-	8.3 (100)	18.8 (100)	
Inocybe rimosa	-	-	0.4 (25)	4.4 (100)	
Lactarius hepaticus	-	-	0.6 (50)	2.7 (75)	
Russula cf *amydagloides*	-	-	0.2 (25)	1.7 (100)	
Russula brevipes	-	-	-	1.3 (75)	
Russula cessans	-	-	2.1 (100)	7.5 (100)	
Russula cf *placita*	-	-	0.4 (25)	1.3 (100)	
Russula puellaris	-	0.2 (25)	1.0 (75)	1.0 (100)	
Tricholoma myomyces	-	-	0.8 (75)	1.0 (75)	
Satellite species					
Albatrellus ovinus	0.2 (25)	0.8 (50)	-	-	
Amanita constricta	-	-	-	1.0 (50)	
Amanita pachycolea	-	-	-	0.2 (25)	

Catethelasma imperialis	-	-	0.4 (25)	-	
Clavaria purpurescens	-	-	-	0.2 (25)	
Cortinarius cf *amurceus*	-	0.4 (25)	-	-	FJ039627
Cortinarius balteatus	0.2 (25)	0.2 (25)			FJ039613
Cortinarius causticus	0.2 (25)	0.4 (50)	-	0.2 (25)	FJ039636
Cortinarius cf *comptulus*	-	-	0.2 (25)	-	
Cortinarius cf *decoloratus*	-	0.2 (25)	-	-	
Cortinarius delibutus	-	0.2 (25)	0.6 (50)	-	
Cortinarius eburneus	-	0.2 (25)	-	-	
Cortinarius fulvochrascens	-	-	0.4 (25)	0.2 (25)	FJ039689
Cortinarius glaucopus	-	-	1.3 (25)	-	FJ039616
Cortinarius humolens	-	-	-	0.2 (25)	FJ039640
Cortinarius infractus	-	0.4 (25)	-	0.2 (25)	FJ039612
Cortinarius cf *largus*	-	-	0.8 (25)	-	
Cortinarius limonius	-	0.4 (50)	0.4 (25)	0.4 (25)	FJ039667
Cortinarius melanotus	-	0.2 (25)	-	-	
Cortinarius mucosus	0.4 (25)	-	-	-	FJ039581
Cortinarius multiformis	-	0.8 (50)	0.2 (25)	0.4 (25)	FJ039635
Cortinarius nanceiensis	-	-	0.2 (25)	-	FJ039670
Cortinarius papulosus	0.2 (25)	1.3 (50)	-	-	FJ039669
Cortinarius percomis	-	0.2 (25)	-	-	FJ039657
Cortinarius pseudoglaucopus	-	-	0.2 (25)	0.2 (25)	FJ039641
Cortinarius cf *rheubarbarinus*	0.2 (25)	-	-	-	
Cortinarius rufo-olivaceous	-	-	0.2 (25)	-	FJ039645
Cortinarius salor	-	-	-	0.2 (25)	FJ039600
Cortinarius scutulatus	0.2 (25)	0.2 (25)	-	0.2 (25)	
Cortinarius talus	-	0.2 (25)	-	0.6 (50)	
Cortinarius cf *turibulosus*	-	0.4 (50)	-	-	FJ039673
Cortinarius cf *variecolor*	-	0.4 (25)	0.2 (25)	0.2 (25)	FJ039633
Cortinarius cf *variegatus*	-	-	-	0.2 (25)	
Cortinarius violaceus	-	0.2 (25)	-	1.3 (50)	FJ039649
Cortinarius viridipes	-	0.2 (25)	0.2 (25)	0.4 (25)	FJ039683
Hydnellum aurantiacum	-	0.2 (25)	-	-	
Hydnellum scrobiculatum	-	-	0.2 (25)	-	

Hygrophorus hyacinthinus	-	-	1.3 (25)	0.4 (25)
Hygrophorus monticola	-	0.8 (25)	0.2 (25)	-
Hygrophorus odoratus	-	0.4 (25)	0.2 (25)	-
Inocybe albodisca	-	-	1.0 (25)	1.0 (50)
Inocybe geophylla var lilacina	-	-	0.4 (50)	0.2 (25)
Inocybe griseolilacina	-	-	-	0.4 (50)
Lactarius circellatus	-	-	-	0.2 (25)
Lactarius luculentus	-	-	-	2.5 (50)
Lactarius olympianus	-	-	-	1.9 (50)
Lactarius resimus	0.4 (50)	-	-	-
Lactarius cf *olivinus*	-	-	0.4 (25)	-
Limacella illinata	-	-	-	0.2 (25)
Polyzellus multiplex	-	-	0.4 (25)	-
Russula abietina	0.8 (25)	0.2 (25)	-	1.3 (50)
Russula borealis	-	0.2 (25)	0.2 (25)	0.2 (25)
Russula cf *integra*	0.2 (25)	-	-	-
Russula cf *consobrina*	0.2 (25)	-	-	-
Russula foetens	-	-	2.3 (25)	2.1 (25)
Russula paludosa	-	0.6 (25)	2.7 (25)	0.4 (25)
Russula parazurea	-	0.2 (25)	-	-
Russula unknown sp	0.2 (25)			
Thaxtergaster pingua	-	-	0.6 (50)	-
Tricholoma flavovirens	0.2 (25)	0.2 (25)	-	-
Tricholoma intermedium	-	-	0.2 (25)	-
Tricholoma pessundatum	-	0.4 (25)	1.0 (50)	0.6 (25)
Tricholoma portentosum	0.4 (25)	-	-	-
Tricholoma sulphureum	-	-	0.4 (25)	-
Tricholoma virgatum	-	-	1.0 (25)	0.2 (25)
Tricholoma unknown sp.	0.4 (25)	-	-	-

4. Distribution and diversity of terrestrial mosses, liverworts and lichens along productivity gradients of a southern boreal forest

Introduction

The forest cryptogam community (mosses, liverworts and lichens) provides a potentially useful benchmark suite of species to gauge anthropogenic effects on forest biodiversity and ecosystem integrity (Frego, 2007). For example, many cryptogam species are sensitive to the changes in environmental conditions and habitat loss from timber harvesting, and patterns in species distribution can provide insights into the maintenance or re-establishment of climax forest conditions (e.g. Fenton and Frego, 2005; Nelson and Halpern, 2005; Hylander et al., 2005). There are also clear differences in cryptogam communities across unaltered landscapes, presumably due to variations in site factors such as humidity, edaphic characteristics and substrate availability (Robinson et al., 1989; Carleton, 1990; Pharo and Beattie, 1997), which greatly contribute to beta-level species diversity. Given this complexity, Frego (2007) emphasized the need for better information from reference undisturbed forests to improve our knowledge of species-specific ecological tolerances and desired endpoints of forest integrity.

Cryptogam surveys are often directed over very large areas encompassing climatic zones or biomes to highlight the differences in primary community assemblages along forest landscapes (La Roi and Stringer, 1976; Schofield, 1988; Belland, 2005). At a much finer scale, a close relationship between cryptogam communities and site properties has been reported through correlations of diversity with variables such as soil texture or vascular plant cover (Pharo et al., 1999; Saetersdal et al., 2003; Vanderpoorten and Engels, 2003). Patterns in cryptogam guild distribution generally follow edaphic gradients as well, with moss and liverwort diversity increasing with soil moisture availability as lichen diversity decreases (Pharo and Beattie, 1997). Diversity indices and the abundance of key species could therefore change abruptly in complex landscapes, and inherent site differences only metres apart could potentially obscure the interpretation of forest management effects via these indicators.

Accounting for the association of cryptogam indicator species with inherent site features within landscapes would therefore be an essential component of effective and accurate forest stand monitoring. In British Columbia, the provincial biogeoclimatic

ecosystem classification describes a logical progression of benchmark sites based on plant associations that reflect gradients in soil moisture and nutrient regimes (Pojar et al., 1987). In most of the provincial forest classification, however, the majority of lichen, moss and liverwort species were infrequent and of low abundance, and were not comprehensively evaluated due to their relatively minor biomass and difficulties in identification. We undertook complete cryptogam surveys of well-recognized plant associations encompassing the widest range in productivity of a southern old-growth boreal landscape to explore the association of soil productivity with terrestrial moss, liverwort, and lichen distributions. Plot size was limited to 0.15 ha to ensure soil moisture/nutrient regimes and related site features were entirely homogenous and consistent among replicates, and sites were chosen within a localized landscape to minimize macroclimate effects on cryptogam distribution.

Our hypothesis in this study was that cryptogam species distribution would strongly reflect site, while diversity overall would not differ due to the replacement of lichens by bryophytes as sites progress from dry-poor to moist-rich (i.e. Robinson et al., 1989; Pharo and Beattie, 1997). We also noted how substrate types (forest floor, coarse woody debris [CWD], surface cobbles, and tip-up mounds) differed between plant associations and explored whether this habitat distribution could be a factor in species composition and diversity. The results of this study will provide insight into the sensitivity of cryptogam communities to site features that may need to be recognized in forest stand monitoring, as well as provide detailed information on old-growth terrestrial cryptogam communities that will facilitate the definition of desired endpoints for ecosystem stewardship.

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 of the province, 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* x *Picea*

engelmannii [Moench] Voss) and subalpine fir (Bl) (*Abies lasiocarpa* [Hook.] Nutt.). 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 (54º49'N 127º10'W; elevation 522 m). Smithers has a mean annual air temperature of 3.9ºC and mean annual precipitation of 513 mm (354 mm as rainfall) (1960-1990; Environment Canada). 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.

Five transects, each with one replicate of each site series, were located along a 25 km portion of the McDonnell Forest Service Road (54º40' to 47'N and 127º16' to 36'W) at approximately 900 m elevation. The study was limited to 19 plots because we were unable to find a suitable Sx – Devil's club plot on the fourth transect. Each plot was 50 m x 30 m (0.15 ha) in size. Plots were separated by a minimum 50 m within each transect. All plots had climax coniferous forests, and were mostly multicohort due to gap-phase disturbances caused by bark beetles, root pathogens and wind throw, as well as a small amount of partial harvesting (ranging from 0-10% of the basal area) that occurred throughout the valley in the 1950s.

The old-growth forests (~ 180 years) on our sites had ceased height growth (i.e. reached an asymptote) decades earlier, and we used the asymptotic or 'maximum obtainable' stand height as a measure of site potential (Ryan and Yoder, 1997). Additional forest stand attributes and soil properties of the study plots were published previously (Kranabetter et al., 2007) as summarized in Table 1. Briefly, the in situ buried bag soil incubation was initiated June 5-9, 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. This was repeated at 5 random microsites per plot. After 5 weeks, the bags were retrieved and

gently run through a 5 mm sieve, followed by an analysis of dissolved organic N and inorganic N. Gravimetric soil moisture content (w/w) of the forest floor and mineral soil (0-20 cm depth) was measured every 3 weeks from mid May to early September. Forest floors (F, H horizons and buried wood) were sampled with a 15 cm diameter template to the mineral soil interface, and mineral soils were sampled to a 20 cm depth using a stony soil auger (4 cm in diameter). Three random microsites were sampled and bulked together per plot, and different microsites were chosen on each sample day. Available light in the understory was assessed at 5 microsites within each plot using hemispherical canopy photographs (set at a 1 m height) using a Nikon Coolpix 5000 camera with a Nikon FC-E8 fisheye converter lens. Any nearby understory trees or shrubs were tipped back, away from the lens, to allow for a measure of light levels reaching the ground surface. The growing season light availability (direct + diffuse light sources from May 15 to September 15), was expressed as a percentage of full sun and was computed from each photograph using the Gap Light Analyser (GLA) 2.0 software, following Frazer et al. (2000).

Forest substrate measures

Coarse woody debris (CWD) was defined as dead woody material in various stages of decomposition, not incorporated into the soil, and larger than 7.5 cm in diameter. CWD did not include stumps, snags, or partially uprooted live trees. Coarse woody debris was sampled using the line intercept method (Van Wagner, 1982). Two 24 m transects were initiated near the plot centre; the first line followed a random compass bearing and the second at the same bearing plus 90 degrees (British Columbia Ministry of Forests, 1998). Diameter was measured perpendicular to the bole at the intersection of the transect and the CWD. Species and the decay class were also noted for each piece. The latter were defined as follows: decay class 1 – intact wood, with bark, elevated on support points; 2 – intact wood, bark removed, sagging slightly; 3 – hard wood but decaying, sagging near ground, some invading roots in sapwood; 4 – decayed with blocky wood texture, resting on ground, roots in heartwood; and 5 – well decayed with friable wood texture, partly sunken into ground; roots in heartwood). Tip-up mounds are formed by fallen trees that leave a small pit and exposed root mat with subsoil (Beatty and Stone,

1986). The number of tip-up mounds was determined through a visual survey of each plot.

Cryptogam community assessment

Each plot was first examined for the most abundant (> 1% ground cover) terrestrial cryptogams, with an estimate of percent cover, as is routinely undertaken in ecosystem classification (British Columbia Ministry of Forests, 1998). Next, a timed one-hour search was undertaken to record the less abundant species, which included collecting specimens to provide voucher material and to identify unknown species at a later date. In addition to the forest floor habitat (humus layer and leaf/needle litter), we focused our search on coarse woody debris, tip-up mounds, and surface cobbles (rocks 7.5 to 25 cm in diameter). Epiphytic cryptogams on live trees, shrubs or standing snags were not included, nor were saxicolous (rock-dwelling) species on bedrock or large boulders (a large rock surface was present only a very small portion of one plot). To document the limitations in species distribution by substrate types, we counted occurrences on forest floor habitat first, coarse woody debris second, surface cobbles third and tip-up mounds last. No attempt was made to estimate percent cover for each species recorded in the timed search.

Bryophytes were identified using Lawton (1971), Schofield (2002), Paton (1999), Damsholt (2002), and the Flora of North America (2007). Lichen specimens were identified using Goward et al. (1994), Goward (1999), and Brodo et al. (2001). Nomenclature follows Anderson et al. (1990) for the mosses, Stotler and Crandall-Stotler (1977) for the liverworts, and Esslinger and Egan (1995) for the lichens. Vouchers of each species were deposited in the British Columbia Forest Service Herbarium in Smithers, B.C.

Statistical analyses

Analysis of cryptogam communities was based on species presence, rather than by relative abundance, as it was not possible to accurately measure percent cover for each species across the entire plot area. We described species richness in two ways, following Newmaster et al. (2003): species richness within plot (alpha diversity, α) and total species within plant association (gamma diversity, γ). Jackknife 1 estimates of total species

richness by plant association and by all sites combined were calculated with EstimateS (Version 7.5) software (Colwell, 2005).

The study was organized in a randomized incomplete block design, with transects treated as blocks. Alpha diversity for all cryptogam species and by the three guilds (moss, liverwort and lichen) were 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 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 (GLM) procedure in SAS using Type 1 Sums of Squares was used to test linear and curvilinear regressions between plot means of dependent and independent variables (n = 19).

A comparison of cryptogam communities among plots was undertaken by Bray - Curtis ordination with PC-ORD 5.0 software using the Sorenson (Bray-Curtis) distance measure (McCune and Grace, 2002). Separation of cryptogam communities by plant association was tested in pairwise comparisons using the multi-response permutation procedure with the Sorenson (Bray-Curtis) distance measure (McCune and Grace, 2002). Indicator species analysis was undertaken with the Monte Carlo technique of Dufrêne and Legendre, using 5000 randomizations and a significance *p* value of < 0.10 (McCune and Grace, 2002).

Results

Stand and soil characteristics

Increasing soil moisture and nitrogen availability across plant associations were associated with both taller trees and greater stand volumes (basal area) (Table 1). These forest stand attributes led to corresponding reductions in light availability, from 29% to 17% of full sun in the forest understory, on average (Table 1). Both the amount and size of CWD increased with stand productivity as well (Table 1), ranging from a low of 12 m^3 ha^{-1} and 8 cm diameter on a poor-Cladonia site to a maximum of approximately 400 m^3 ha^{-1} and 50 cm diameter on a very rich – Devil's club site. Decay class 5 comprised 40% of the CWD volume across all sites, followed by approximately 19% each for classes 2, 3 and 4, while fresh CWD (decay class 1) contributed 3% of the total. The number of tip-

up mounds ranged from 2 to 16 per plot, and were slightly more common on medium - Huckleberry sites (Table 1).

Terrestrial cryptogam community

Total terrestrial cryptogam richness of the study sites (2.85 ha in sum) was 148 taxa (47 mosses, 23 liverworts, and 78 lichens), of which 30% were uncommon (found on only 1 plot) (Appendix 1). Cryptogam community composition (all species presence/absence) aligned consistently with plant associations, with significant separation among poor – Cladonia, medium – Huckleberry and rich – Oak fern sites (Fig. 1). A small number of species dominated in abundance, with only 11 species contributing 2% or greater ground surface cover on at least one site (Table 2). Forest floor substrate hosted slightly less than half (43%) of the cryptogam species, with the remainder of the community limited in distribution to either coarse woody debris (35%), surface cobbles (14%) or tip-up mounds (8%).

Trends in species richness between cryptogam guilds corresponded with site productivity, as characterized in these plots by asymptotic stand height. Lichen richness declined with greater stand height, while moss and liverwort richness increased (Fig. 2a). We noted that liverwort richness tended to plateau in correlations with stand height, but the trend was not strong enough to warrant a nonlinear curve (Fig. 2a). The distribution of cryptogam species also varied by substrate across productivity gradients: cryptogam species richness on the undisturbed ground surface (forest floor and cobbles) declined with stand height, while species richness increased with productivity on the more isolated habitat provided by coarse woody debris (Fig. 2b). Tip-up mounds provided an additional 5 species per plot, on average, which did not change significantly among plant associations ($p = 0.745$).

With contrasting responses between lichens and bryophytes (mosses and liverworts), as well as by substrates, the α diversity for all terrestrial cryptogams was quite consistent at 39 taxa per plot (0.15 ha) among poor, rich and very rich sites, with a significant reduction in α diversity detected only for medium-richness sites (Table 3). Overall, however, we did not detect a linear or curvilinear relationship between cryptogam α diversity and asymptotic stand height (Fig. 3a). In contrast, vascular plant α diversity (unpublished plot data; W. MacKenzie) was positively correlated to stand

height, which paralleled the pattern in bryophyte richness ($r^2 = 0.74$), but not cryptogam richness overall ($p = 0.620$).

Total species richness by plant association (γ diversity) was approximately twice that of α diversity due to the high number of uncommon species (Table 3), demonstrated by the cumulative species curves (Fig. 4). Unlike α diversity, we found a peak in γ diversity on rich - Oak fern sites (Table 3). Jackknife 1 estimates of total species richness also indicated the greatest diversity would be found on rich - Oak fern sites, followed by poor-Cladonia and very rich-Devil's club sites, with the lowest diversity on medium-Huckleberry sites (Table 3). The species richness of this landscape, based on all 19 plots, was estimated by jackknife 1 analysis to be 191 species.

A total of 23 cryptogam species were found at least once in each of the four plant associations (7 mosses, 5 liverworts, 11 lichens) (Appendix 1). Some of the more abundant generalist species of these southern boreal stands include *Dicranum fuscescens*, *Pleurozium schreberi*, *Ptilium crista-castrensis*, *Ptilidium pulcherrimum*, *Cladonia coniocraea*, *Cladonia ochrochlora*, *Parmeliopsis ambigua*, *Parmeliopsis hyperopta*, and *Peltigera aphthosa*. A total of 30 species (9 mosses, 2 liverworts, 19 lichens) were consistent and frequent enough in distribution to significantly characterize plant associations (Appendix 1). Species more abundant on dry, poor forests include *Dicranum polysetum*, *Cladina mitis*, *Cladina rangiferina*, *Cladonia uncialis*, *Nephroma arctica* and *Stereocaulon tomentosum*, while rich to very rich sites were characterized by the abundance of *Mnium spinulosum*, *Plagiomnium insigne*, *Rhizomnium magnifolium* and *Rhizomnium nudum*. Two liverwort species (*Barbilophozia hatcheri*, *Eremontus myriocarpus*) were indicative but not exclusive to mesic sites.

Discussion

The detailed survey of cryptogam species from these plots in northwest British Columbia revealed communities comparable to similar surveys of other northern coniferous forests. Species richness of terrestrial cryptogams averaged approximately 38 species per plot and 70-80 species for a study area in central British Columbia (Botting and Fredeen, 2006), while in montane forests Pharo and Vitt (2000) reported 36 species on average for stands and 90 species overall for bryophytes and lichens. Our total of 148 taxa was somewhat higher than other studies, possibly reflecting the wide gradient in

sites and our inclusion of the saxicolous crust lichens that are often overlooked. The relatively high number of infrequent cryptogam species within a stand dominated in cover by a few common species (especially feathermosses) is also quite typical for many coniferous ecosystems (La Roi and Stringer, 1976).

This small-scale, detailed study of cryptogam communities presents a general model of guild (moss, liverwort and lichen) distribution and species richness that we expect to be consistent with many boreal and montane landscapes. Terrestrial cryptogam community composition was well aligned with plant associations, and this relationship emphasized the linkage between species distribution and ecosystem attributes related to soil productivity. Key environmental variables for cryptogam distribution, such as light availability and air humidity (Robinson et al., 1989; Mills and MacDonald, 2005) were strongly interrelated with soil productivity and corresponding stand attributes, and within these old-growth boreal landscapes we found asymptotic stand height to be the simplest integrator of these covarying influences. For the purposes of a monitoring program, it is important to recognize that a wide range in site productivity can be found within short distances in these complex landscapes, and that a mosaic of plant associations such as the ones surveyed here can be found within a typical forest cutblock.

In addition to environmental variables, there were important substrate characteristics, especially the volume and diameter of CWD (Rambo and Muir, 1998; Mills and MacDonald, 2004), linked to soil and stand productivity. Without the additional surface area provided by CWD, for example, the cryptogam diversity of rich and very rich sites would have been largely reduced to only those leafy mosses occupying the forest floor. Some of the reduction in cryptogam species on mesic sites could perhaps be attributed to microsite limitations via smaller diameter CWD inherent to moderately productive forests (Kruys et al., 1999). Surface cobbles were also an important substrate and could be found on all site types, but the reduced plant vigour on poor sites provided an opportunity for saxicolous crustose lichens to establish, in contrast to richer sites where cobbles were overgrown by feathermosses.

The positive correlation between vascular plant and bryophyte (moss and liverwort) richness with site productivity was consistent with previous studies in boreal forests (Jonsson and Jonsell, 1999; Saetersdal et al., 2003), and lichens supplemented

total cryptogam richness to a large extent on poor, dry sites as we had hypothesized. Nevertheless, some reduction in combined species richness on mesic sites (30% compared to rich sites, both in α and γ diversity) was evident for the three guilds. Carleton (1990) concluded that cryptogam species were best adapted to contrasting conditions at either ends of fertility gradients, and we also noted only 15% of the cryptogam community in this landscape could be considered generalists (i.e. widely tolerant to site type). Mesotrophic sites were generally characterized by the lack of cryptogam species adapted to the dry-poor and moist-rich ends of the fertility spectrum (saxicolous/terricolous lichens and epixylic bryophytes, respectively), and it was these specialist species that had the most utility as indicators of undisturbed forest site conditions (Appendix 1).

The results of this study also emphasize the value of contrasting ecosystems encompassing wide edaphic gradients for the conservation of cryptogam diversity. Protected areas that included poor and rich plant associations with mesic sites in this boreal landscape would more than double terrestrial cryptogam richness (from 64 species on mesic sites vs. 139 species combined). In addition, intensive harvesting and short rotations would produce forest stands with low volumes of CWD and few tip-up mounds, which would be expected to result in reduced cryptogam diversity (Jonsson and Esseen, 1990; Crites and Dale, 1998; Rambo, 2001), especially in lush, productive sites. Every site type contained some uncommon species, as has often been reported (e.g. Vitt et al., 2003), and we suspect their distribution reflects the rich mosaic of microsites in old-growth forests (Cleavitt, 2005; Gignac and Dale, 2005), along with some influence of stochastic processes involving dispersal and establishment.

More detailed study of mechanisms responsible for species distribution, such as drought tolerance or competitive interactions (Frego and Carleton; 1998; Sulyma and Coxson; 2001), would strengthen these findings, along with further surveys by substrate type to test hypotheses related to habitat specificity. For example, most of the CWD in these old-growth stands was well decomposed (decay class 4 and 5) and hosted the majority of cryptogam species; less decayed wood may host fewer species, but exploring this substrate limitation further would require a separate inventory of cryptogams from each piece of CWD. Another observation worth further investigation is whether

cryptogam species shift substrates with environmental conditions, and could be, for example, exclusively terrestrial or epixlyic depending upon site. We noted a number of species occupying more than one substrate type, but as yet have not definitively established where habitat changes in response to site conditions.

In conclusion, terrestrial cryptogam communities of old-growth coniferous forests were linked to soil fertility, as characterized by vascular plant associations or asymptotic stand height, presumably due to the combined resource availability of light, moisture, nutrients and substrate types. The relationship to soil fertility was most notable in the distribution of species within guilds (lichens versus bryophytes) and by substrate types (forest floor and cobble habitat versus CWD). Soil fertility effects associated with α and γ diversity indices were more subtle, and were primarily indicated by reductions in species richness on mesic sites. Only a small number of generalist cryptogam species were found across these site types, and the majority of species were more limited in distribution, although often infrequent and of low abundance as well. As an indicator group for ecosystem assessment, terrestrial cryptogam communities should be referenced to plant associations (or a similar characterization of site) with benchmarks of diversity indices and species distributions established from climax forests.

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Table 1. Forest stand and soil characteristics among four plant associations across a productivity gradient (mean, with SE in brackets).

Plant association*	Asympototic stand height (m)	Forest basal area (m^2 ha^{-1})	Soil N availability (kg ha^{-1})	Soil moisture (kg m^{-2})	Available sunlight (%)	CWD volume (m^3 ha^{-1})	CWD diameter (cm)	Tip-ups mounds (# per ha)
P – Cladonia	21a (1.4)†	36a (2)	17.6a (2.7)	13.4a (1.2)	29a (0.8)	22a (4)	13.2a (1.3)	4.2a (0.8)
M – Huckleberry	28b (0.5)	58b (5)	30.2b (1.9)	18.7a (1.5)	20b (0.6)	165b (51)	15.4a (1.0)	10.2b (1.9)
R – Oak fern	32c (0.4)	70b (6)	40.8c (2.0)	29.3b (2.1)	18bc (0.6)	232b (41)	20.7b (1.7)	7.4ab (0.7)
VR – Devil's club	36d (0.7)	104c (4)	46.7c (4.3)	27.6b (2.0)	17c (0.6)	274b (82)	23.3b (1.7)	6.0a (0.7)

* soil nutrient regimes 'P' poor, 'M' medium, 'R' rich, 'VR' very rich

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

Table 2. Abundance of dominant terricolous cryptogam species across plant associations. Values are % groundcover with % frequency in brackets.

Cryptogam species	Poor – Cladonia (n = 5)	Medium – Huckleberry (n = 5)	Rich – Oak fern (n = 5)	Very Rich – Devil's club (n = 4)
Mosses				
Pleurozium schreberi	79% (100)	43% (100)	35% (100)	20% (100)
Ptilium crista-castrensis	0.2% (20)	32% (100)	30% (100)	12% (100)
Hylocomium splendens	0	7% (80)	12% (100)	0.5% (25)
Dicranum fuscescens	1% (100)	5% (80)	1% (20)	3% (25)
Plagiomnium insigne	0	0	3% (40)	7% (100)
Rhizomnium nudum	0	0	0	3% (50)
Rhytidiadelphus triquestris	0	0.1% (20)	1% (40)	2% (50)
Timmia austriaca	0	0	0.5% (40)	3% (25)
Liverworts				
Barbilophozia lycopodioides	0.1% (20)	0.5% (60)	0.1% (40)	0.5% (25)
Lichens				
Cladina mitis	5% (100)	0	0	0
Cladina rangiferina	5% (100)	0	0	0
Cladonia ecmocyna	2% (80)	0	0	0
Peltigera aphthosa	0.5% (80)	0.1% (40)	0.1% (20)	0
Nephroma arctica	0.5% (60)	0	0	0

Table 3. Species richness of moss, liverwort and lichen guilds by plant association (mean number of species per 0.15 ha plot, with SE in brackets)

Plant association*	Moss species	Liverwort species	Lichen species	α diversity	γ diversity	Jackknife estimate
P – Cladonia	9a (0.7)†	3a (0.4)	28a (0.6)	40a (0.9)	78	94 (3.2)
M – Huckleberry	10a (0.9)	7b (0.7)	12b (1.9)	29b (2.1)	64	86 (5.7)
R – Oak fern	15b (0.6)	10b (0.6)	15b (2.1)	40a (2.7)	89	122 (5.9)
VR – Devil's club	16b (0.8)	8b (1.1)	12b (1.1)	37a (1.2)	72	99 (3.9)

Note: γ diversity for very rich sites was determined from 4 plots rather than 5.

* soil nutrient regimes 'P' poor, 'M' medium, 'R' rich, 'VR' very rich

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

Fig. 1. Bray-Curtis ordination of cryptogam communities (all species presence/absence). Axis 1 = 34% and axis 2 = 13% of the total variance. Selected pairwise *p* values: poor vs. medium = 0.002; medium vs. rich = 0.002; rich vs. very rich = 0.666.

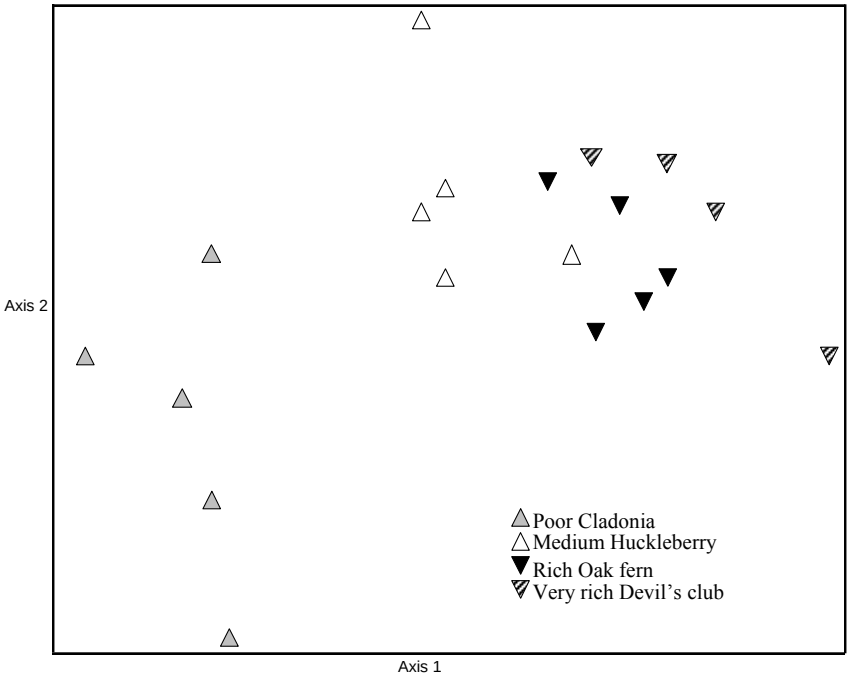
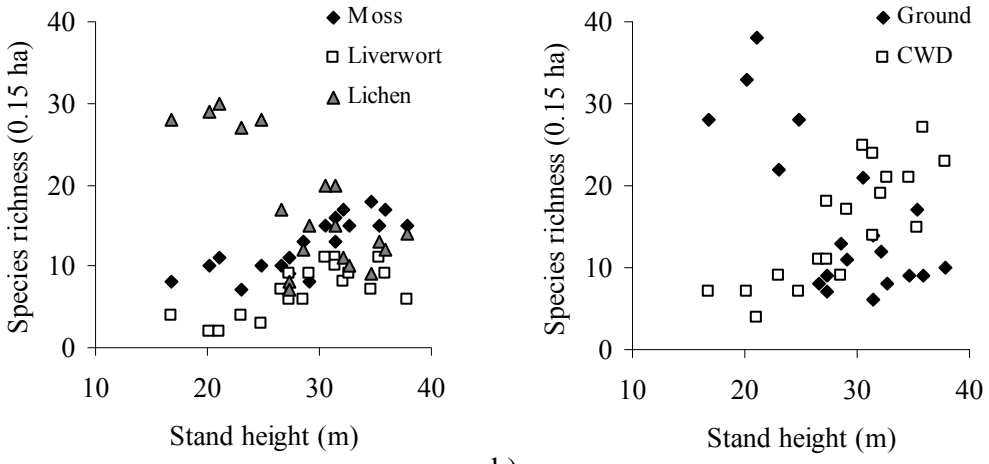


Fig. 2. Terrestrial cryptogam species richness per plot (0.15 ha) as a function of asymptotic stand height for a) moss, liverwort and lichen guilds, and b) substrate ('ground' = forest floor and surface cobbles).



a) b)

Fig. 3. Alpha (α) diversity (0.15 ha) for terrestrial cryptogams by plant association across stand height.

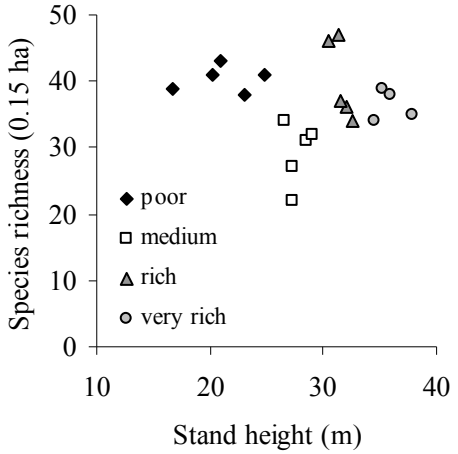
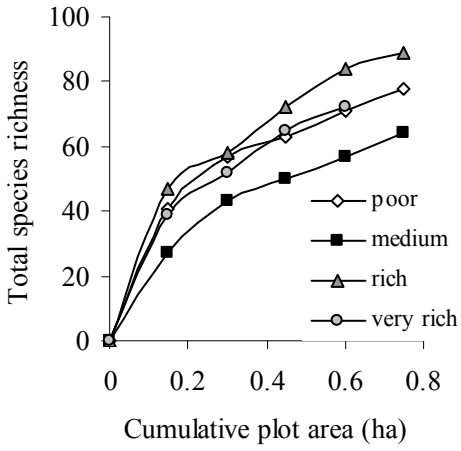


Fig. 4. Cumulative species richness (γ diversity) across replicates (0.75 ha, except very rich sites at 0.60 ha) for the four plant associations.



Appendix 1. Terrestrial cryptogam species distribution (% frequency) across the four plant associations. Species in bold have significant indicator value for characterizing plant associations ($p < 0.10$ with Monte Carlo analysis). Habitat distribution summarized for all plots; F = forest floor; R = rock; W = coarse woody debris; T = tip up mound.

Cryptogam species	Poor – Cladonia (n = 5)	Medium – Huck.berry (n = 5)	Rich – Oak fern (n = 5)	V. Rich – Devil's cl. (n = 4)	Habitat (FRWT)
Mosses					
Amblystegium serpens	-	-	20	25	W,T
Andreaea rupestris	80	-	-	-	R
Brachythecium albicans	-	20	80	75	F,W,T
Brachythecium asperrimum	-	20	20	25	F,T
Bryum capillare	-	-	20	-	T
Buxbaumia aphylla	-	20	-	-	T
Buxbaumia piperi	-	-	20	-	W
Buxbaumia viridis	-	-	20	-	T
Campylium chrysophyllum	-	-	20	-	T
Ceratodon purpureus	60	20	-	25	F,T
Dicranella hetermalla	20	20	-	-	T
Dicranoweisia crispula	40	-	-	-	R
Dicranum fuscescens	100	100	100	100	F,W
Dicranum polysetum	80	60	20	-	F
Dicranum scoparium	40	40	60	50	F,R,W,T
Dicranum tauricum	-	20	-	50	W
Distichium inclinatum	-	-	20	-	T
Eurhynchium pulchellum	-	-	40	25	W,T
Hygrohypnum dilitata	-	-	-	25	F
Hylocomium splendens	40	80	100	25	F
Hypnum circinale	-	-	40	50	W
Isopterygium pulchellum	-	-	20	25	W
Mnium spinulosum	-	20	80	100	F,W,T
Orthotrichum striatum	-	-	-	25	T
Plagiomnium cuspidatum	-	-	-	25	F

Plagiomnium insigne	-	20	60	100	F,W,T
Plagiothecium denticulatum	-	60	60	100	F,W,T
Pleurozium schreberi	100	100	100	100	F
Pohlia nutans	80	100	100	75	F,W,T,R
Polytrichum alpinum	-	-	20	50	F
Polytrichum commune	-	20	-	-	F
Polytrichum juniperinum	100	40	40	25	F,T
Polytrichum piliferum	20	-	-	-	F
Pseudoleskeella nervosa	-	-	20	-	R
Ptilium crista-castrensis	40	100	100	100	F
Racomitrium heterostichum	60	-	-	-	R
Rhizomnium magnifolium	-	-	80	25	F,T
Rhizomnium nudum	-	-	20	100	F,W
Rhytidiadelphus loreus	-	-	20	-	F
Rhytidiadelphus triquetrus	-	20	80	75	F,W
Rhytidiopsis robusta	20	40	40	-	F
Sanionia uncinata	-	20	40	75	W
Scouleria aquatica	20	20	-	25	F
Tayloria serrata	20	20	-	25	F
Tetraphis pellucida	-	-	20	50	W
Tetraplodon mnioides	20	-	-	-	F
Timmia austriaca	-	20	20	50	F,T
Liverworts					
Anastrophyllum hellerianum	-	-	60	50	W
Barbilophozia hatcheri	80	100	60	25	F,R,W,T
Barbilophozia lycopodioides	20	80	80	50	F
Blepharostoma trichophyllum	-	20	100	100	W
Calepogeia mulleriana	-	-	20	-	T
Calepogeia suecica	-	-	20	-	W
Cephalozia lunulifolia	-	80	60	50	W
Eremontus myriocarpus	-	60	20	-	W
Jamesoniella autumnalis	-	-	40	50	W
Leicolea alpestris	-	-	-	25	W
Lepidozia reptans	-	-	60	25	F,W

Lophozia capitata	-	-	20	25	T
Lophozia guttulata	-	100	100	100	W,T
Lophozia incisa	-	40	60	75	W
Lophozia longidens	20	80	80	75	W,T
Lophozia obtusa	-	-	20	-	W
Marchantia polymorpha	-	-	20	-	T
Plagiochila porelloides	-	-	20	25	F,W
Pleuroclada albescens	20	-	-	-	T
Ptilidium californicum	60	40	20	25	R,W,T
Ptilidium pulcherrimum	80	100	100	100	F,W,T
Scapania bolanderi	-	40	-	25	W
Unknown liverwort	-	-	20	-	W
Lichens					
Amandinea punctata	20	-	-	-	F
Baeomyces placophyllus	20	-	-	-	T
Baeomyces rufus	40	40	20	-	F,T
Bellemerea alpina	20	-	-	-	R
Bellemerea cinereorufescens	80	-	-	-	R
Bellemerea subsorediza	60	-	-	-	R
Biatorella sp.	-	20	-	-	R
Catinaria atropurpurea	-	-	20	-	W
Cetraria ericetorum	60	-	20	-	F,W
Chaenotheca furfuracea	20	40	80	50	W,T
Cladina mitis	100	-	-	-	F
Cladina rangiferina	100	-	-	-	F
Cladina stellaris	20	-	-	-	F
Cladonia bellidiflora	60	-	-	-	F,T
Cladonia carneola	40	40	20	25	W
Cladonia cenotea	80	-	60	25	F,W,T
Cladonia chlorophaea	40	-	40	25	F,W,R
Cladonia coniocraea	40	60	60	100	W
Cladonia cornuta	80	-	-	-	F
Cladonia deformis	80	60	-	-	F,W
Cladonia ecmocyna	80	-	-	-	F

Cladonia fimbriata	-	-	20	25	W
Cladonia gracilis	60	-	-	-	F
Cladonia multiformis	100	-	40	-	F,W,R
Cladonia norvegica	20	20	40	-	W
Cladonia ochrochlora	40	80	80	75	W,T
Cladonia parasitica	-	-	20	-	W
Cladonia prolifica	20	60	20	-	W,T
Cladonia pyxidata	40	-	-	-	F,T
Cladonia umbricola	-	20	-	-	W
Cladonia uncialis	100	-	-	-	F
Cyphelium inquinans	-	-	40	-	W
Lecanora intricata	40	-	-	-	R
Fuscopannaria praetermissa	-	-	-	25	W
Lecanora polytropa	80	-	-	-	R
Lepraria incana	-	20	40	50	W, T
Lobaria halleri	-	-	-	25	W
Lobaria pulmonaria	-	-	-	25	W
Mycoblastus sanguinarius	-	-	40	25	W
Nephroma arctica	80	20	-	-	F
Nephroma bellum	-	40	20	75	W
Nephroma parile	-	-	40	25	W
Nephroma resupinatum	-	-	20	25	W
Ochrolechia arborea	-	20	-	-	W
Ochrolechia gowardii	20	-	-	-	W
Parmeliopsis ambigua	100	100	100	100	W
Parmeliopsis hyperopta	100	100	100	100	W
Peltigera aphthosa	100	80	100	50	F,W,T
Peltigera bacilliformis	20	-	-	-	W
Peltigera canina	40	20	40	25	F,W,T
Peltigera chionophila	40	20	20	-	F,R
Peltigera didactyla	40	-	-	-	F,T
Peltigera horizontalis	20	20	40	25	F,W,T
Peltigera leucophlebia	40	40	40	25	F,T
Peltigera malacea	60	20	20	-	F,W,T

Peltigera membranacea	-	-	80	75	F,W,T
Peltigera neopolydactyla	20	20	80	100	F,W,T
Peltigera polydactylon	-	20	-	50	W,T
Peltigera praetextata	-	40	40	25	F,W,T
Peltigera venosa	-	-	20	25	F,T
Pertusaria pupillaris	-	40	20	-	W
Placynthiella dasaea	-	-	20	-	F
Placynthiella sp.	-	20	-	-	F
Platismatia glauca	-	-	20	-	W
Porpidia cinereoatra	80	-	-	-	R
Porpidia contraponenda	60	-	-	-	R
Porpidia speirea	40	60	20	-	R
Protothelenella corrosa	20	-	-	-	R
Psoroma hypnorum	-	-	20	-	W
Rhizocarpon geographicum	80	-	-	-	R
Rhizocarpon hochstetteri	80	-	-	-	R
Stereocaulon tomentosum	80	20	-	-	F,R,T
Trapeliopsis granulosa	20	-	-	-	F
Umbilicaria deusta	20	-	-	-	R
Umbilicaria hyperborea	60	-	-	-	R
Vulpicida pinastri	20	-	-	-	W
Xylographa vitiligo	20	20	-	-	W

5. Soil meso- and macro-faunal communities along productivity gradients of southern boreal forests

The final stages of taxonomic resolution are near completion for this project, and so only preliminary data analysis has taken place. We have identified a large number of faunal species from the pitfall traps and Tullgren funnel extractions: 47 species of springtails; ~ 115 species of mites; 66 species of spiders; 12 species of ants; ~ 25 species of rove beetles; and many dozen Carabid, millipede and centipede specimens are still being worked on. Compared to the previous taxa, the preliminary assessments show much less distinct community organization of soil fauna with regard to soil fertility, and many species are either rare or widely distributed. Presumably this is because of the relatively constant habitat (bryophytes, acidic humus and decayed wood) of the soil surface across all site types, along with the greater mobility of fauna. Therefore we would suggest the effects of vegetation (coniferous vs deciduous) or climate (interior continental vs coastal temperate) to be more influential on species composition than either soil nutrient or moisture regime effects within a landscape. Some relationship between species composition and the morphology of forests floors, especially mor to moder humus types, was hypothesized, but no clear patterns emerged. Instead any effect of fauna might be manifested by the activity of the populations, especially during the dry part of the summer, and the form of the predominant litter types (herbaceous leaves > conifer needles > bryophytes).

6. Contrasts among mycorrhizal guilds in foliar nitrogen concentration and ^{15}N discrimination along productivity gradients of a boreal forest.

Introduction

Individual plant communities with characteristic floristic composition and structure are well described by many systems of forest site classification, both at broad climatic scales and along landscapes with variable topography and gradients in soil moisture and fertility (Kimmins 1997). One possible mechanism underpinning the distribution of floral communities was presented by Read and colleagues (Read 1994, Read et al. 2004), who suggested climax vegetation was characterized by the predominance of plants with a particular type of mycorrhizal symbiosis. These fungal mutualisms provide differing abilities to access growth-limiting resources, especially nitrogen (N), with a key adaptation identified for mycorrhiza capable of facilitating organic N release by proteolytic activities for direct uptake. Under this hypothesis, forest ecosystem classification systems and the distribution of major mycorrizal guilds (arbuscular, ericoid and ectomycorrhizal fungi; Peterson et al. 2004) would reflect primarily the degree of soil impoverishment, and the reliance on specialized mechanisms for N acquisition from recalcitrant litter sources (Kielland 1994, Giesler et al. 1998, Björk et al. 2007). At issue with this hypothesis, however, is that in some investigations both labile and recalcitrant organic N uptake have not been limited to any one mycorrhizal guild, nor even strictly mycorrhizal plants (Näsholm et al. 1998, Hawkins et al. 2000, Hodge et al. 2001, Rains and Bledsoe 2007), raising questions as to how closely linked these fungal mutualisms are to soil fertility.

In British Columbia (Canada), forest classification with the biogeoclimatic system has been underway for nearly 3 decades, and although the distribution of mycorrhizal guilds has not been explicitly tested, the general patterns in understory plant composition is supportive of a fundamental mycorrhizal effect on floristic community organization. Xeric to mesic, N-poor sites are often dominated by understory shrub species of the order Ericales with ericoid mycorrhiza, such as *Vaccinium* and *Gaultheria*. Ericoid mycorrhiza produce very specialized lateral 'hair' roots that can tolerate low soil pH and utilize recalcitrant organic matter such as chitin as a source of N. In contrast, moist, rich soils with high rates of N mineralization and nitrification are typically dominated in the

understory by a rich assemblage of arbuscular fern and angiosperm species. Arbuscular mycorrhizas have intracellular hyphae and arbuscles within the root cells, and appear to be particularly well adapted to NO_3^-, along with NH_4^+ and simple amino acids (Nordin et al. 2001). The majority of conifer and broadleaf tree species of these boreal and montane forests have ectomycorrhizal fungal symbionts. Ectomycorrhizal fungi are noteworthy for their species diversity, with likely dozens of species colonizing a single tree, and these complex fungal communities provide an array of abilities to access perhaps both organic and inorganic N sources (Nygren et al. 2007; Cajsa et al. 2008) across a range of site types (Toljander et al. 2006, Kranabetter et al. 2009). Amongst the remaining more minor mycorrhiza guilds are the arbutoid fungi, which are taxonomically aligned with ectomycorrhizal fungi but form morphologically distinct structures on root systems (Peterson et al. 2004). Arbutoid fungi are found on a handful of herb species of northern forests, including *Pyrola*, *Orthilia* and a woody shrub, *Arctostaphylos*.

Evidence for direct organic N uptake by mycorrhiza is increasingly conclusive (Whiteside et al. 2009), but demonstrating the differences in N cycles and uptake among a broad selection of plants and mycorrhizal guilds in unaltered forests is challenging. One avenue for investigating N cycles and plant nutrition is natural isotope ratios of ^{14}N to ^{15}N ($\delta^{15}N$) but interpretations are not straightforward, however, as factors such as the degree of N retention by mycorrhiza (Hobbie and Colpaert 2003), species effects in isotopic fractionation (Emmerton et al. 2001), variation in soil substrates (Högberg 1997) or differences in rooting depth (Schulze et al. 1994) can potentially influence foliar ^{15}N values. Given these limitations, perhaps the best application of these tools would be in a replicated, 'common garden' design along an edaphic gradient of contrasting soil fertility, where leaf N concentration ($N_{\%}$), leaf ^{15}N and the relative slope of correlations with soil N indices could be compared directly amongst mycorrhizal guilds.

In this study we present foliar $N_{\%}$ and ^{15}N data from understory species of old-growth boreal forests along natural gradients in soil productivity. The productivity gradient was characterized by a shift in N quantity and quality, from predominantly low amounts of strictly organic N to progressively greater rates of N mineralization with site potential (Kranabetter et al. 2007). Two plant species from four mycorrhizal guilds (arbuscular, ectomycorrhiza, ericoid and arbutoid) were sampled to compare inter and

intraspecific differences in $N_{\%}$ and ^{15}N discrimination along the soil N gradient. We hypothesized that the mycorrhizal guilds associated with organic N uptake (ericoid, ectomycorrhiza, and arbutoid) would show a stronger response in foliar nutrition and ^{15}N discrimination on the low productivity sites, in contrast to the arbuscular species better adapted to highly productive soils. The objective of the study was to provide more field-based evidence for the underlying effect of mycorrhiza guild on the floristic composition of late-seral forests.

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* x *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 (54º49'N 127º10'W). 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.

Study design

Five blocks were located along a 25 km portion of the McDonnell Forest Service Road (54º40' to 47'N and 127º16' to 36'W) at approximately 900 m elevation. Mean annual air temperature of these sites is estimated, based on ClimateBC extrapolation (Spittlehouse 2006) at 2.3ºC, 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 (4 plant associations x 5 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 x 30 m (0.15 ha) in size. The forests were old growth (~ 180 years) and mostly multicohort due to gap-phase disturbance caused by bark beetles, root pathogens and wind throw, as well as a small amount of partial harvesting (ranging from 0-10% of the basal area) that occurred through the valley in the 1950's. Further descriptions of stand, soil and vegetation characteristics of the study plots are listed in Kranabetter et al. (2007).

Plot properties and plant communities

A survey of the understory vascular plant community was undertaken over a 20 x 20 m subplot for the shrub layer (woody plants 0.15 – 10 m in height, including regenerating conifers) and herb layer (all herbaceous species and low woody plants less than 15 cm tall). Percent cover by each species was estimated visually based on the percentage of ground surface covered by the crowns when projected vertically (LMH 25).

Total nitrogen content and in situ N availability (Table 1) were assessed for the soil profile (forest floor and 0-20 cm mineral soil) in 2006 at each plot as described in Kranabetter et al. (2007). Briefly, three bulked subsamples of forest floor and mineral soil were collected from each plot, air-dried, sieved to remove coarse fragments, and measured for total N by combustion elemental analysis. Dissolved organic N, NH_4^+ and NO_3^- were determined from a 5 week in situ incubation initiated in late spring. Forest floor F and H horizons were sampled as intact cores, 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 persulphate solution, and forest floor and mineral soil N concentration data was converted to mass per ha using depth and coarse fragment content values from each plot.

Foliar sampling

Sampling took place in the fall (Sept. 13-15) of 2008. Leaves and needles were sampled from 10 plant species of the understory encompassing 4 mycorrhizal guilds: ectomycorrhiza (advanced regeneration approximately 1.5 m in height of *A. lasiocarpa*

and *P. glauca* x *englemanii*); ericoid mycorrhiza (*Vaccinium membranaceum* and *Menziesia ferruginea*), arbutoid mycorrhiza (*Orthilia secunda* and *Pyrola asarifolia*), and arbuscular mycorrhiza (*Linnaea borealis, Paxistima myrsinites, Rubus parviflorus* and *Viburnum edule*). The four arbuscular plant species included both evergreen (*L. borealis*, *P. myrsinites*) and deciduous leaf habit (*R. parviflorus*, *V. edule*). Mycorrhizal associations were confirmed through literature searches. Three to five lateral shoots lateral shoots of the current year's growth were clipped from 5 individual plants per species and bulked into one sample per species per plot. In a few cases where a species was at very low abundance (e.g., *V. edule* on mesic sites) we were limited to fewer subsamples than five for the plot. Approximately thirty to fifty epigeous sporocarps of ectomycorrhizal fungi that were deemed fresh (near button stage) and larval-free were collected from each plot and bulked into a single sample. Each plot collection contained 10 – 15 species of ectomycorrhizal fungi.

Petioles were removed from the leaves before drying at 70°C for 24 hours. Sporocarps were cleaned with a damp rag to remove any adhering soil or needle litter, and 5 – 7 g of fresh material per species was set aside (either as several small sporocarps of a single species or a portion removed from large sporocarps) to create a balanced bulk sample of species for drying. The dried foliage and fungal tissue was ground to ? μ and analyzed for total N by dry combustion with the Leco CHN-600 analyzer (Kalra and Maynard 1991). The natural abundance of δN^{15} isotope was determined using AS autosampler of a Thermoquest (Carlo Erba Instruments) NC 2500 elemental analyzer. The mass ratio 29/28 of the sample relative to the mass ratio 29/28 of a nitrogen reference gas was used to determine the isotopic value of the sample. Lab standards are calibrated against the international standards IAEA-N1 (+0.4 per mil) and IAEA-N2 (+20.3 per mil). Nitrogen isotopic values are listed relative to air $\delta^{15}N$ vs air (Hauck et al. 1994). We also included one subsample of understory *A. lasiocarpa* per plot for analysis from a 2006 study of these same sites (Kranabetter and Simard 2008) to test sample year effects on foliar $N_{\%}$ and $\delta^{15}N$.

Statistical analysis

Foliar and soil parameters were tested against plant association or mycorrhizal guild in a randomized incomplete block design with five replicate transects (blocks). We

used Proc Mixed in SAS (SAS Inc. 2004) with block and block interactions set as random factors. Significant differences between least square means of each plant association were tested using pairwise *t* tests at a significance level of 0.05. Regression analysis using the GLM procedure in SAS was used to examine relationships between foliar attributes and the independent variable of soil N availability. Block and block interactions were tested and never found to be significant in the regressions.

Results

A total of 76 vascular plant species (27 and 39 species in the shrub and herb layers, respectively) were found in the forest understories, with the majority (45) having arbusuclar mycorrhiza. There were 7 ectomycorrhizal species (or predominantly so, as some species reportedly have both ECM and arbuscular mycorrhiza), 5 ericoid species, 6 arbutoid species and 4 species with orchid mycorrhiza. The species richness and % cover of arbuscular plants in the understory increased with soil N availability, while that of ectomycorrhiza, ericoid and arbutoid species collectively declined (Fig. 1).

The natural ^{15}N abundance of forest floors and upper mineral soils differed by 3.7‰ ($p < 0.001$), on average, but neither substrate differed significantly among the plant associations ($p = 0.979$ and $p = 0.781$ for forest soils and mineral soils, respectively) (Table 1). A possible effect of sample year on foliar parameters was tested by comparing *A. lasiocarpa* collected in 2006 and 2008. There were no differences detected in foliar $N_{\%}$ ($p = 0.241$) or $\delta^{15}N$ ($p = 0.256$) between years along the edaphic gradient.

Foliar N concentrations generally increased with soil N availability, but with a significant interaction detected between mycorrhizal guilds (Table 2). Eight of the 10 plant species had a postive linear or curvilinear (*L. borealis*) correlation between foliar $N_{\%}$ and soil N availability, with the sharpest increases in $N_{\%}$ noted for the deciduous arbuscular plants (*R. parviflorus*, *V. edule*) (Table 2, Fig. 2). The ectomycorrhizal species of *A. lasiocarpa* and *P. glauca* were the only pair to also demonstrate an intraguild interaction (Table 2); *A. lasiocarpa* had improved N nutrition on the poor to mesic sites in comparison to *P. glauca* (Fig. 2). Nitrogen concentrations of the bulked EMF sporocarps was also positively correlated to soil N availability.

The natural abundance of ^{15}N in foliage strongly differed between mycorrhizal guilds, with a significant interaction as well between guilds in correlation with soil N

availability (Table 2). Both ectomycorrhizal species were less depleted in ^{15}N along the N gradient, ranging from approximately -6‰ on the poorest soils to -1.5‰ on the richest sites (Fig. 3). An interaction was detected between these species as well, consistent with foliar $N_{\%}$ patterns, with *P. glauca* more depleted in ^{15}N than *A. lasiocarpa* on the poor to mesic sites. In contrast, a weak negative correlation ($p < 0.10$ with one outlier removed) in ^{15}N was found for the bulk EMF sporocarps (Fig 3), which were far more enriched in ^{15}N than the trees (average of +5.9‰). Similar to ectomycorrhizal trees, a positive trend in foliar $\delta^{15}N$ with soil N availability was found for the ericoid *V. membranaceum*, but this species ranged from -3‰ on the poorest soils to +2‰ on the richest sites (Fig. 3, Table 2). In strong contrast to the previous guilds were the arbutoid species; *P. asarifolia* had a negative correlation in foliar $\delta^{15}N$ with soil N availability, ranging from +8‰ on the poorest soils to -1‰ on the richest sites, while *O. secunda* had a weak, positive correlation across the productivity gradient that averaged +4.5‰ overall. Lastly, the foliar $\delta^{15}N$ of the arbuscular plants was in comparison poorly correlated to soil N availability; no relationship was detected for *P. myrsinites* or *R. parviflorus*, while *L. borealis* and, to a less degree, *V. edule* ($p < 0.10$), had a slight positive increase in $\delta^{15}N$ along the productivity gradient (Table 2, Fig. 3).

Discussion

The discussion is in preparation for this manuscript and will be completed in the spring of 2009.

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Table 1. N content and availability indices of the soil profile (forest floor and 0-20 cm mineral soil), and δN^{15} abundance of bulk forest floor and mineral soil among four plant associations comprising the productivity gradient (mean and SE in brackets).

Plant association*	n	Total N (kg ha^{-1})	DON (kg ha^{-1})	NH_4^+ (kg ha^{-1})	NO_3^- (kg ha^{-1})	FF δN^{15} (‰)	Min δN^{15} (‰)
P – Cladonia	5	961a (72)	16.7a (2.7)	0.9a (0.2)	0a	0.35 (0.2)	4.1 (0.2)
M – Huckleberry	5	1343b (125)	27.1b (1.6)	3.2b (1.0)	0a	0.33 (0.2)	4.0 (0.3)
R – Oak fern	5	2535c (264)	33.1b (1.4)	7.5c (1.0)	0.2b (0.1)	0.49 (0.3)	4.4 (0.3)
VR – Devil's club	4	3513d (224)	32.0b (3.3)	9.2c (3.6)	5.5c (3.3)	0.49 (0.3)	3.8 (0.4)

* soil nutrient regimes 'P' poor, 'M' medium, 'R' rich, 'VR' very rich

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

Table 2. Regression analysis of species foliar N concentration and δN^{15} abundance in correlation with soil N availability

	n	Foliar $N_{\%}$ ($g\ kg^{-1}$)	Foliar δN^{15} (‰)
All plant species			
Mycorrhizal guild	5	$p = 0.070$	$p = < 0.001$
Soil N availability		$p = < 0.001$	$p = 0.069$
Guild x soil N		$p = 0.041$	$p = < 0.001$
Ectomycorrhiza			
Abies lasiocarpa	19	$= 8.8 + 0.10(kg\ N)$; $r^2 = 0.73$	$= -7.1 + 0.10(kg\ N)$; $r^2 = 0.73$
Picea glauca	14	$= 5.9 + 0.18(kg\ N)$; $r^2 = 0.72$	$= -7.5 + 0.0025(kg\ N)^2$; $r^2 = 0.78$
Species x soil N		$p = 0.035$	$p = 0.037$
Ericoid			
Vaccinium membranaceum	17	$= 12.3 + 0.13(kg\ N)$; $r^2 = 0.46$	$= -3.3 + 0.09(kg\ N)$; $r^2 = 0.44$
Menziesia ferruginea	9	$p = 0.241$	$p = 0.406$
Species x soil N		$p = 0.999$	$p = 0.240$
Arbutoid			
Orthilia secunda	16	$= 10.3 + 0.17(kg\ N)$; $r^2 = 0.56$	$= 1.8 + 0.08(kg\ N)$; $r^2 = 0.38$
Pyrola asarifolia	14	$p = 0.064$	$= 10.9 – 0.23(kg\ N)$; $r^2 = 0.75$
Species x soil N		$p = 0.398$	$p = < 0.001$
Arbuscular (evergreen)			
Linnaea borealis	18	$= 5.3 + 0.35(kg\ N) – 0.0038(kg\ N)^2$; $r^2 = 0.63$	$= -4.0 + 0.06(kg\ N)$; $r^2 = 0.27$
Paxistima myrsinites	12	$= 9.5 + 0.15(kg\ N)$; $r^2 = 0.47$	$p = 0.671$
Species x soil N		$p = 0.629$	$p = 0.176$
Arbuscular (deciduous)			
Rubus parviflorus	17	$= 10.2 + 0.24(kg\ N)$; $r^2 = 0.76$	$p = 0.474$
Viburnum edule	13	$= 8.3 + 0.23(kg\ N)$; $r^2 = 0.50$	$p = 0.083$
Species x soil N		$p = 0.695$	$p = 0.148$
Ectomycorrhizal fungal sporocarps	19	$= 26.8 + 0.18(kg\ N)$; $r^2 = 0.36$	$p = 0.094$; $r^2 = 0.17$

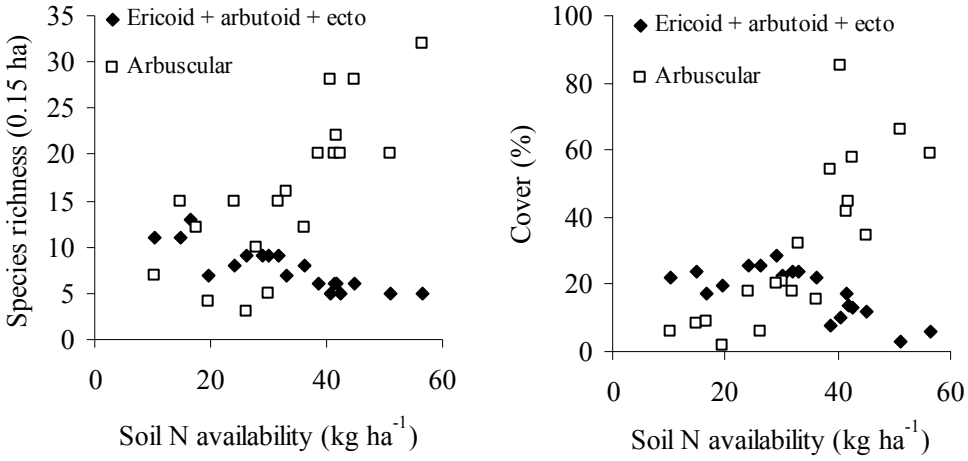
Species richness (0.15 ha)
Ericoid + arbutoid + ecto
Arbuscular
Soil N availability (kg ha-1)
Cover (%)
Ericoid + arbutoid + ecto
Arbuscular
Soil N availability (kg ha-1)

Fig. 1

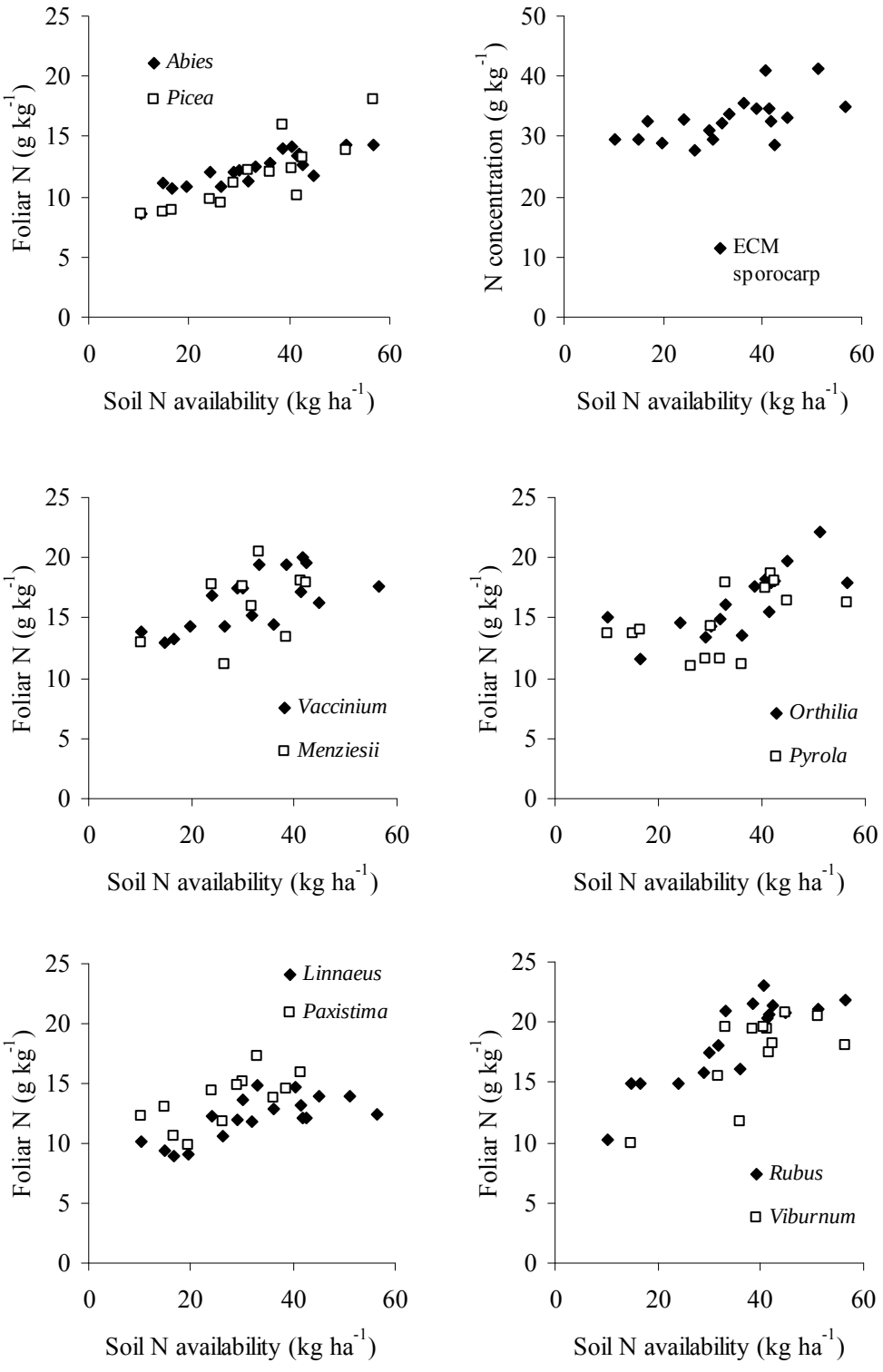
Foliar N (g kg-1)
Soil N availability (kg ha-1)
Abies
Picea
N concentration (g kg-1)
ECM sporocarp
Vaccinium
Menziesii
Orthilia
Pyrola
Linnaeus
Paxistima
Rubus
Viburnum

Fig. 2

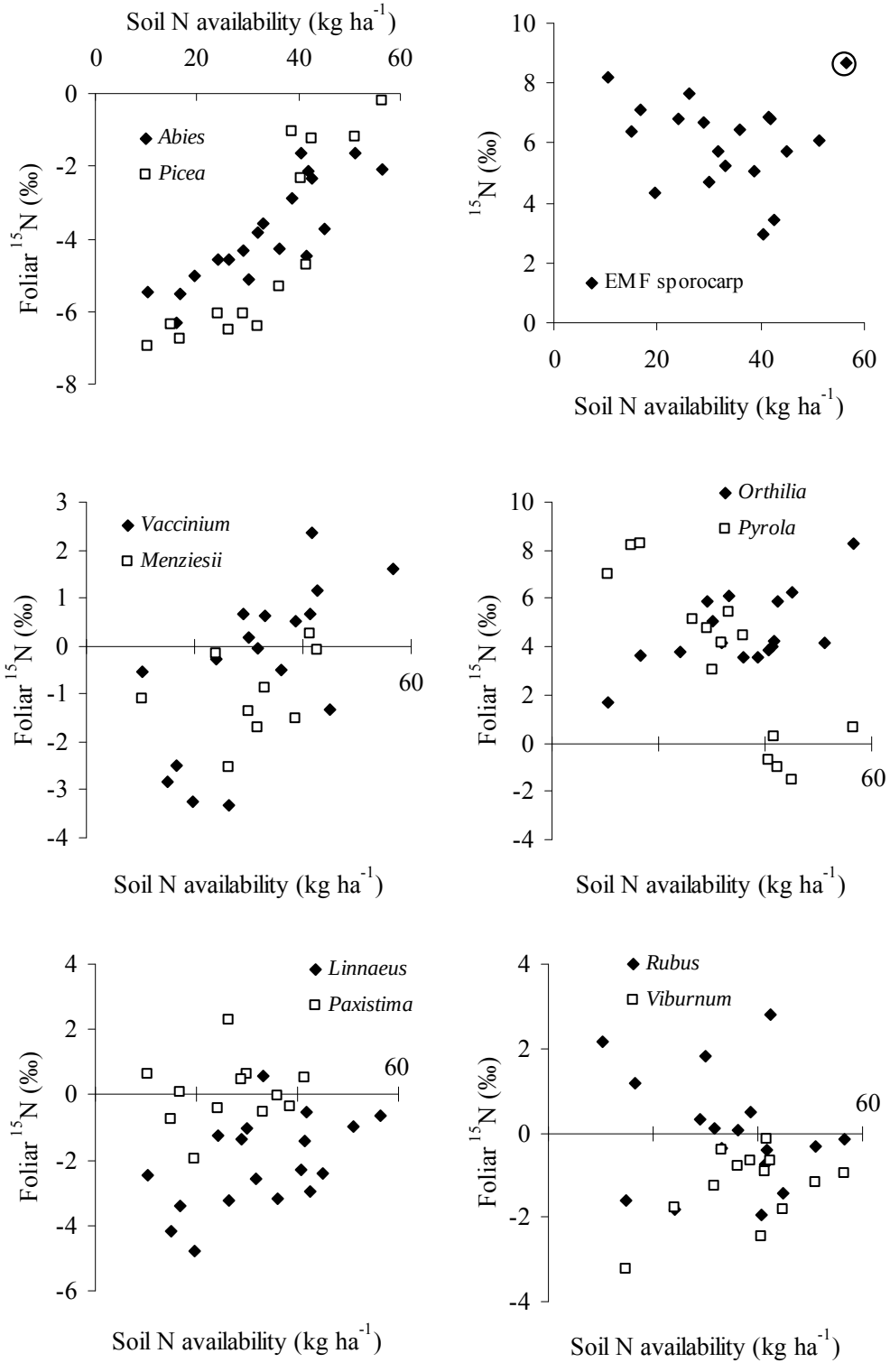
Soil N availability (kg ha-1)
Foliar 15N (‰)
Abies
Picea
EMF sporocarp
Vaccinium
Menziesii
Orthilia
Pyrola
Linnaeus
Paxistima
Rubus
Viburnum
60

Fig. 3

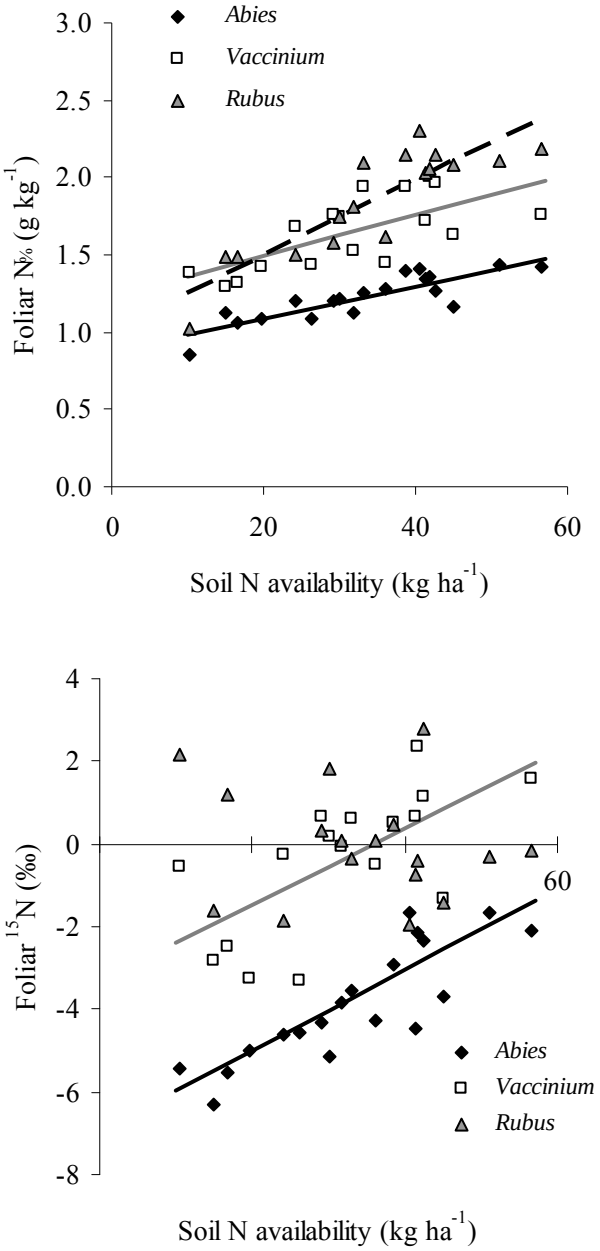
Abies
Vaccinium
Rubus
Foliar N‰ (g kg-1)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
20
40
60
Soil N availability (kg ha-1)
Foliar 15N (‰)
4
2
0
-2
-4
-6
-8
60
Abies
Vaccinium
Rubus
Soil N availability (kg ha-1)

Fig. 4

Appendix. Average cover (%) of understory vascular plant species, separated into shrub and herb layers, with associated mycorrhizal guild. Frequency in brackets.

Species	Guild	Poor – Cladonia (n = 5)	Medium – Huck.berry (n = 5)	Rich – Oak fern (n = 5)	V. Rich – Devil's cl. (n = 4)
Shrub layer					
Abies lasiocarpa	EMF	4.2	15.6	12.6	4.8
Picea glauca x englemanii	EMF	1.3	1.1	2.0	0.5
Pinus contorta	EMF	2.2	-	-	-
Alnus viridis	EMF/AM	2.0	13.0	5.8	5.3
Amelanchier alnifolia	EMF/AM	0.3	0.1	0.1	-
Salix scouleriana	EMF/AM	1.2	-	-	-
Shepherdia canadensis	EMF/AM	3.0	1.5	-	-
Menziesia ferruginea	ErM	5.0	0.8	1.1	-
Vaccinium membranaceum	ErM	7.0	5.8	0.9	0.1
Vaccinium ovalifolium	ErM	0.2	0.1	0.2	0.2
Acer glabrum	AM	-	-	-	2.0
Cornus stolonifera	AM	-	-	0.5	-
Juniperus communis	AM	0.1	-	-	-
Lonicera involucrata	AM	-	0.2	0.6	1.8
Oplopanax horridus	AM	-	-	1.0	16.0
Paxistima myrsinites	AM	0.6	0.9	1.0	0.5
Ribes lacustre	AM	-	0.1	0.9	2.8
Ribes laxiflorum	AM	-	-	-	2.0
Rosa acicularis	AM	0.3	0.1	0.1	0.2
Rubus idaeus	AM	-	-	-	0.1
Rubus parviflorus	AM	0.1	0.3	3.0	3.3
Rubus spectabilis	AM	-	-	-	0.1
Sambucus racemosa	AM	-	-	-	0.1
Sorbus scopulina	AM	0.2	0.5	0.1	-
Sorbus sitchensis	AM	-	0.1	0.1	-
Symphoricarpos albus	AM	-	-	-	0.2
Viburnum edule	AM	-	0.2	0.5	0.5

Herb layer					
Arctostaphylos uva-ursi	EEM	3.3	0.2	-	-
Chimaphilia umbellata	EEM	0.7	0.3	-	-
Moneses uniflora	EEM	-	0.2	0.5	0.1
Orthilia secunda	EEM	-	0.5	0.4	0.1
Pyrola asarifolia	EEM	0.1	0.3	1.4	2.8
Pyrola chlorantha	EEM	0.1	0.3	0.2	-
Empetrum nigrum	ErM	0.2	-	-	-
Vaccinium caespitosum	ErM	1.1	-	0.5	-
Corallorhiza trifida	Orchid	-	-	-	0.1
Goodyera oblongifolia	Orchid	0.1	0.1	0.1	-
Listera borealis	Orchid	-	-	0.1	-
Listera cordata	Orchid	-	0.2	0.1	-
Actaea rubra	AM	-	-	0.2	0.4
Arnica cordifolia	AM	0.2	0.3	0.5	-
Athyrium filix-femina	AM	-	-	0.1	0.2
Calamagrostis canadensis	AM	-	-	-	1.0
Clintonia uniflora	AM	0.2	0.6	0.8	6.3
Cornus canadensis	AM	4.0	9.2	8.0	6.3
Diphasiastrum complanatum	AM	0.7	-	-	-
Dryopteris expansa	AM	-	-	0.1	1.7
Epilobium angustifolium	AM	0.2	-	-	0.1
Equisetum arvense	NM	-	-	0.1	-
Galium boreale	AM	0.1	-	0.1	-
Galium triflorum	AM	-	-	0.1	0.1
Geocaulon lividum	AM	0.4	0.1	0.1	-
Gymnocarpium dryopteris	AM	-	0.1	11.4	18.0
Heracleum maximum	AM	-	-	0.1	0.2
Lathyrus nevadensis	AM	-	-	0.3	0.4
Linnaea borealis	AM	1.0	2.2	1.1	0.1
Lycopodium annotinum	AM	-	0.2	0.9	0.3
Lycopodium clavatum	AM	0.1	-	-	-
Maianthemum racemosum	AM	-	0.1	0.2	0.2
Maianthemum stellatum	AM	-	-	4.1	1.0

Mitella nuda	AM	-	-	1.0	-
Oryzopsis asperifolia	AM	0.1	-	-	-
Osmorhiza berteroi	AM	-	-	0.1	0.2
Osmorhiza sp.	AM	-	-	-	0.1
Petasites frigidus	AM	-	-	0.3	-
Rubus pedatus	AM	-	4.6	9.8	6.3
Rubus pubescens	AM	-	-	0.2	0.1
Sanguisorba canadensis	AM	-	-	-	0.5
Schizachne purpurascens	AM	0.2	-	0.1	-
Streptopus amplexifolius	AM	-	0.1	0.4	0.8
Streptopus roseus	AM	-	-	1.2	1.0
Thalictrum occidentale	AM	-	-	-	0.2
Tiarella trifoliata	AM	-	-	1.0	3.3
Trisetum canescens	AM	-	0.1	1.6	0.5
Veratrum viride	AM	-	0.2	-	0.1
Viola adunca	AM	-	-	0.1	-

EMF = ectomycorrhiza; ErM = ericoid mycorrhiza; EEM = ectendomycorrhiza (arbutoid); AM = arbuscular mycorrhiza; NM = nonmycorrhizal

7. Conclusions: biodiversity of the southern boreal forest across productivity gradients

In planning for biodiversity, forest managers could benefit from further insights into the importance of 'site', that is, the relative uniqueness of species assemblages within a site series in relation to the entire landscape. Our studies suggest a few conclusions along this line of inquiry, and this discussion will be updated and finalized with the completion of the faunal studies. The number of species per plot (0.15 ha) ranged from 145 to 205 (vascular plants, cryptogams, ectomycorrhizal fungi, springtails, spiders and ants), which represents about 1/3rd the total diversity of this landscape (607 species; not including beetles, mites). Total species richness by plot increased by approx. 33% and then plateaued with site productivity (Fig. 1), suggesting that greater amounts of soil moisture, nutrients and organic matter allowed for the development of a richer community of organisms. Beta diversity, which is the turnover of species across a landscape, averaged 31% by plot, meaning any one individual plot had approx. 30% of the total species found over the 19 plots. The uniqueness of species assemblages by site series was moderate, and so, for example, preserving mesic sites alone would capture about 50% of the total landscape organisms. Capturing a range of dry to moist upland sites as oldgrowth management areas or wildlife reserves would therefore potentially double the total number of species within the conservation areas. Lastly, our results should be considered in monitoring programs for late-seral species, which generally use space for time substitution as an experimental design. Such an approach would be less site dependent for fauna, as the majority of these species were ubiquitous, whereas cryptogams and ectomycorrhizal fungi could be confounded by site effects on species assemblages. The benefit of this site sensitivity, however, is that these species provide better indicators for ongoing changes in soil fertility under forest management. Overall we found a large source of biodiversity associated with forest soils and substrates (6 x the diversity of the vascular plants), and a large pool of potential species for environmental monitoring.

Fig. 1. Total species richness of vascular plants, terrestrial cryptogams, ectomycorrhizal fungi, springtails, spiders, and ants by plot in correlation to stand productivity (asymptotic height).