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

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Summary

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

45 Keywords: ectomycorrhizal fungal community, nitrogen, boreal forests, species richness,
46 diversity-productivity relationships, ectomycorrhizal exploration type.

Introduction

Ectomycorrhizal (ECM) fungi are the key mediating agent between soils and trees, and research into these diverse communities continues to expand upon the abiotic - biotic relationships fundamental to forest ecology. These investigations include the association of ECM communities with edaphic and climatic factors (Gehring *et al.*, 2006), the role of ECM species and fungal networks in forest nutrition and productivity (Paul *et al.*, 2007; Selosse *et al.*, 2006), and the dynamics of ECM communities in primary or secondary forest succession (Nara, 2006; Twieg *et al.*, 2007). Ultimately the insights into ECM community ecology gained from these lines of inquiry should provide a better understanding of forest soils 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 ECM ecology is the relationship between soil productivity, especially nitrogen (N) supply, and ECM 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 & Näsholm, 2001), and that many ECM fungi of boreal and subalpine forests can facilitate organic N availability and uptake (Chalot & Brun, 1998; Read & Perez-Moreno, 2003). In addition, a number of experimental studies with N fertilizer or of atmospheric N deposition have demonstrated large shifts in ECM species distribution with improved inorganic N availability (Peter *et al.*, 2001; Lilleskov *et al.*, 2002; Avis *et al.*, 2003) and often losses in 'specialist' or stress-tolerant ECM species (Wallenda & Kottke, 1998; Taylor *et al.*, 2000). These results suggest, at least

for conifer species, that the primary niche of ECM fungi is nutrient-poor, acidic organic soils with negligible rates of N mineralization (Read *et al.*, 2004). For these reasons we might expect ECM diversity in coniferous forests to decline with increasing soil N availability (Parrent *et al.*, 2006), 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 a wide array of ECM species able to thrive on N-rich sites (Toljander *et al.*, 2006). Rather than changes in diversity per se, the effect of soil fertility might be revealed through shifts in the distribution of genera such as *Cortinarius* and *Tricholoma* (Trudell & Edmonds, 2004), in the abundance of mushroom fruiting (Kårén & Nylund, 1997; Jonsson *et al.*, 2000), or in the functional attributes suggested by mantle characteristics (Nilsson & Wallander, 2003). Few studies have thoroughly examined ECM 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 (Natel & Neumann, 1992; Gehring *et al.*, 1998; Kernaghan & 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 extent of this edaphic variation and influence on ECM communities appears to be subtle (Kranabetter *et al.*, 2005; Yamashita *et al.*, 2007; B. Twieg, unpublished).

Detailed study of ECM species distribution across well defined and replicated natural edaphic gradients would help clarify the significance of soil fertility to ECM

communities. One such gradient was described for upland plant associations of southern boreal forests in British Columbia (Canada), where increasing stand productivity was indicated by dissolved inorganic and organic N mass, along with strong positive correlations with foliar N concentrations (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 climax ECM communities, and we were able to refine the comparison by sampling a single host species, subalpine fir (*Abies lasiocarpa* [Hook.] Nutt.), which had regenerated throughout these old-growth forests.

In this study, we report on the relationships between natural gradients in soil productivity and ECM fungal communities of *A. lasiocarpa*, including diversity estimates, species distribution and 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 ECM 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.

Five transects 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 transect, generally within a radius < 1 km (4 plant associations x 5 transects = 20 plots). We were unable to find a suitable Sx – Devil's club plot at the fourth transect, 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). Some key site properties published previously are summarized in Table 1 and briefly described below.

Site properties

These old-growth forests (~ 180 years) 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. All plots had mixed coniferous forests but with differences in 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.

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 and inorganic N 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, ranging in height from 1 to 2.5 m, established naturally under the canopy and had been suppressed for some decades. 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 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, Cal.).

Ectomycorrhizal fungal assessment

Roots for ectomycorrhizal fungal 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 ECM fungal 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 randomly selected saplings were sampled 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 saplings were refrigerated and examined immediately, while the remaining 35 saplings 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 randomly selected and the number of root tips colonized by each ECM morphotype was determined. Successive root sections were examined until 200 root tips had been counted from each of the saplings. 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 macroscopically (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 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, depending on the thickness of the mantle layers. 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 collected from the Pacific Northwest (British Columbia Ectomycorrhizal Research Network, 2007). In addition, we characterized the exploration type of each morphotype based on Agerer (2000): '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.

ITS analysis of ectomycorrhizal fungi

DNA information was used to supplement morphotyping for fungi and to clarify the taxonomy of distinct morphotypes. This was especially important for *Cortinarius*, as most of these species were characterized by large populations of bent to tortuous root tips with thick, white emanating hyphae (4-5 μm in diameter with large clamps) and no other notable features.

A subsample (~ 5 root tips) was collected from a total of 96 fungal populations (clusters of root tips with the same morphotype from individual saplings) 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, Canada). The tips were pulverized with a ceramic bead in a FastPrep (FP120) high speed shaker (Thermosavant, Holbrook, USA). After centrifuging briefly, the supernatant was transferred to micro collection tubes provided in the DNeasy Plant Kit. At this point, the instructions of the DNeasy Plant Kit were followed. The genomic DNA was stored at -20°C. Primer pairs used in PCR amplifications were either ITS1F-ITS4B or NSI1-NLC2. Samples were sequenced using the Big Dye Terminator Kit (Applied Biosystems, Foster City USA). 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.

Statistics

ECM fungal 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 ECM community of each plot (5 saplings combined) was determined using PC-ORD 5.0 (McCune & Grace, 2002).

The study was organized in a randomized complete block design, with transects treated as blocks. Species richness and exploration type abundance was tested among plant associations using Proc Mixed in SAS (SAS Inc., 1988) 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 correlations between plot means of dependent and independent variables (n = 19). No significant effect of block or block interactions were found in any of the correlations.

A comparison of ECM 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 & 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 ECM fungal communities by plant association was tested in pairwise comparisons using the multi-response permutation procedure with the Sorenson (Bray-Curtis) distance

measure (presence/absence) (McCune & Grace, 2002). ECM community similarity based on species abundance (% root colonized) was determined by percentage similarity (Pielou, 1984).

Results

We identified a total of 74 ECM fungal taxa through morphotyping and ITS analysis. 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 (30 species) of the most abundant ECM taxa from this community is presented to illustrate contrasts in fungal distribution among plant associations (Table 2).

The number of ECM 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 2). 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. Overall the extent of ECM α diversity increased asymptotically with soil fertility, 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 2), and we were unable to detect significant differences among plant associations ($p = 0.153$). With replicates combined the γ diversity peaked at 41 species on rich Oak fern and very rich – Devils club sites (Table 2), equal to an approx. 20% increase over poor-Cladonia and medium-Huckleberry sites.

The ECM communities showed a progressive separation by plant association in the NMS analysis, along with strong differences in pairwise comparisons of species assemblages between poor, medium and very rich sites (Fig. 2). Pearson and Kendall correlations were most significant between axis 1 and soil N indices, including DIN:DON ratio ($r^2 = 0.758$) and inorganic N mass ($r^2 = 0.803$). Other soil parameters, however, such as soil moisture, pH, and organic P content covaried positively with soil N indices and foliar $N_{\%}$ as well (Table 1), so it was not possible to isolate N as the sole cause of ECM community distribution.

Community similarity analysis also revealed the increasing uniqueness in ECM fungal distribution and abundance with fertility, with as little as 24% overlap in ECM communities between the extreme contrasts in plant associations (Table 3). An intermediate degree of shared ECM species was found between medium–Huckleberry and rich–Oak fern sites (Fig. 2, Table 3), 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 ECM species were evenly distributed across plant associations, and some of the more common ECM species had clear trends in abundance in relation to soil fertility. This was demonstrated for 6 ECM 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 ECM 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 2). The distribution of ECM fungi by

297 exploration type was quite consistent among plant associations, averaging 7 contact, 11
298 short-distance, 14 medium-fringe, 2 medium-mat, and 3 medium-smooth species per plot.
299 The abundance of three exploration types changed significantly with plant association
300 (Fig. 4); short exploration fungi declined on the rich-Oak fern and very rich – Devil's
301 club sites ($p = 0.028$), as medium-fringe and medium-smooth fungi increased ($p = 0.033$
302 and $p = 0.037$, respectively).

303 **Discussion**

304 The significant and quite consistent changes in distribution and abundance of 74
305 ECM species demonstrated a high degree of community specialization along these
306 gradations in soil fertility. The results suggest that ECM species distribution across
307 landscapes, like many vascular and nonvascular forest plants, is largely defined by
308 adaptation and competition for niches related to stress tolerance (i.e. drought, acidity) and
309 resource availability (especially amino acids, NH_4^+ and NO_3^-) in soils (Dickie et al. 2002,
310 Kennedy et al. 2007). Furthermore, the increase in ECM α diversity with soil fertility and
311 foliar $N_{\%}$ confirms the presence of a robust nitrophilic ECM community, even in these
312 cool, moderately-productive boreal landscapes. A reasonable conjecture, from both this
313 and similar results (Gehring *et al.*, 1998; Toljander *et al.*, 2006; Robertson *et al.*, 2006), is
314 that the wide ecological amplitude of *A. lasiocarpa* across such contrasting gradients in
315 soil fertility would depend to some degree on its partnership with these site-specific ECM
316 fungal communities.

317 A hump-backed or unimodal distribution of plant diversity with soil fertility is
318 often proposed (and widely debated) by ecologists, where relatively few plant species are
319 successful on both the most stressful and competitive sites (Mittelbach *et al.*, 2001).

Some parallels can be drawn to this ECM community since there was a clear 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 ECM 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 & 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 ECM 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 & 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 experimentally applied, resulting in more dramatic effects of N fertilizer on ECM 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}). It is also possible that nitrophilic fungal species would take some time

to migrate after N inputs, and consequently only the losses in poorly-adapted ECM species would be observed after experimental treatments. Another consideration, however, is that ECM fungi 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 (Giesler *et al.*, 1998). Perhaps then high amounts of N deposition on mesic sites would be unsuitable for nitrophilic ECM species that cannot tolerate soil drying. Another example of these possible resource combinations was the rich-Oak fern site identified as an outlier (Fig. 1), which had an unusually high forest floor pH of 5.6 among the gradient range of approx. 4.0 to 5.0; this pH level may have had a deleterious effect on some ECM species otherwise adapted to these soil moisture and N conditions. Very likely a few soil properties (moisture, nitrogen, pH etc.) must be aligned to create suitable habitat (Trudell & Edmonds, 2004), and we might consider any perturbation to forest ecosystems with no natural analogue as a determent to ECM fungi.

Among these diverse communities were ECM 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 perhaps of strategic importance that many of these multi-site ECM species are also multi-stage and multi-host fungi, able to persist and thrive in regenerating stands with most tree species (Kranabetter, 2004). The capacity of

366 ECM fungi to buffer disturbances, in a resilience context, might also include severe
367 drought events (Swaty *et al.*, 2004) or more gradual but significant climatic trends (e.g.
368 Pacific Decadal Oscillation) that could affect soil processes and productivity. Along with
369 generalist fungi there were also ECM species more limited in distribution (e.g. *R.*
370 *decolorans*, unknown fungi VI, *T. stuposa*), which are likely well-adapted to specific
371 edaphic niches and contribute to the most complete utilization of soil resources possible.
372 ECM communities may have a degree of functional redundancy, as with many soil biota,
373 but certainly a wide mix of species attributes (multi-site and site-specific species, multi-
374 stage and late-seral species, multi-host and host-specific species) should insure resiliency
375 and sustain productivity in a stressful, dynamic, and unpredictable forest environment
376 (Perry *et al.*, 1989; Loreau, 2000).

377 It is perhaps not surprising there were only minor trends in the distribution of
378 fungal exploration types with soil fertility in comparison to N fertilizer treatments given
379 the relatively subtle shift in N amounts and forms. The consistent mix of mantle types
380 could reflect high functional diversity in response to the heterogeneity of microsites and
381 resources found throughout the fertility gradient (Baier *et al.*, 2006). Presumably ECM
382 fungi would contribute significantly to microbial biomass across the entire productivity
383 gradient, with some effect of rooting density, while shifts in ericoid and arbuscular fungi
384 would correspond to the distribution of understory plants (Nilsson *et al.*, 2005). The
385 visual perception of ECM fungal abundance, as a characteristic of humus forms (Green *et*
386 *al.*, 1993), is more likely a reflection of shifts in ECM communities on sites such as these
387 because conspicuous mat-forming fungi, especially bright yellow *P. fallax*, declined as
388 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 ECM species through root sampling is exceedingly difficult (Taylor, 2002), however, and ongoing sporocarp surveys will help define the distribution of these more infrequent fungi.

Other than poor-Cladonia sites, the difference in α diversity or Shannon's diversity index among plant associations was quite insignificant compared to the profound shifts in ECM 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 ECM species themselves in evaluating forest processes (Wallenda *et al.*, 2000; Dahlberg, 2001). Likewise, it is possible that controlled studies with ad hoc ECM species assemblages could draw incongruous conclusions if ill-suited ECM species were selected for the experimental soil conditions. For example, greenhouse studies of tree nutrition and N forms do not always account for ECM species (e.g. Bennett & Prescott, 2004), which is understandable given the inability to recreate such specialized and complex ECM 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 ECM species need to be considered in testing of tree-soil interactions.

In conclusion, we found strong associations between soil productivity and ECM communities, and confirmed the presence of a diverse array of ECM fungi (both multi-

site and site-specific) inhabiting a spectrum of very poor to very rich edaphic niches. The amount and type of N supply (organic N, NH_4^+ and NO_3^-) was a significant habitat attribute, which, along with soil moisture and pH, would likely define species distribution within this landscape. The significance of such extensive ECM fungal 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. The patterns in ECM communities from these Canadian boreal stands were quite consistent with Swedish forests (Toljander *et al.*, 2006), and we propose that a high degree of habitat specialization is universal, and would contribute to the adaptations required to successfully exploit such vast boreal and temperate landscapes by relatively few tree species. Consequently the simplification of site-adapted ECM communities through anthropogenic activities might hamper the survival of conifers on stressful sites, impede the 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. Site potential (height), understory *A. lasiocarpa* foliar N concentrations, and selected soil parameters by plant association (mean and SE in brackets).

Plant association*	Stand height (m)	Foliar $N_{\%}$ ($g\ kg^{-1}$)	NO_3^- ($kg\ ha^{-1}$)	NH_4^+ ($kg\ ha^{-1}$)	DON ($kg\ ha^{-1}$)	Forest floor pH	Soil moisture ($kg\ m^{-2}$)	Organic P ($kg\ ha^{-1}$)
P – Cladonia	21a† (1.4)	9.7a (0.12)	0a	0.9a (0.2)	16.7a (2.7)	4.0a (0.05)	13.4a (1.2)	137a (8)
M – Huckleberry	28b (0.5)	11.5b (0.17)	0a	3.2b (1.0)	27.1b (1.6)	4.1a (0.07)	18.7a (1.5)	179ab (25)
R – Oak fern	32c (0.4)	12.6c (0.14)	0.2b (0.1)	7.5c (1.0)	33.1b (1.4)	4.7b (0.15)	29.3b (2.1)	246b (24)
VR – Devil's club	36d (0.7)	13.6d (0.17)	5.5c (3.3)	9.2c (3.6)	32.0b (3.3)	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 2. Diversity indices and a partial list (30 species) of the most frequent ectomycorrhizal fungi by plant association (mean and SE in brackets).

	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 (SE)		
Cenococcum geophilum	9.6 (2.3)	16.6 (1.0)	13.7 (2.0)	9.1 (4.9)
MRA	25.6 (7.7)	20.9 (2.5)	9.6 (4.6)	1.0 (0.9)
Unknown fungus VI	10.1 (1.0)	4.0 (2.1)	4.6 (1.4)	1.8 (0.9)
Unknown fungus VIII	8.7 (2.2)	3.3 (1.2)	3.4 (1.3)	3.8 (1.1)
Amphinema byssoides	0.2 (0.2)	2.2 (0.7)	4.7 (3.9)	13.4 (4.4)
Laccaria laccata	1.8 (1.2)	2.8 (1.8)	5.4 (1.1)	9.9 (3.0)
Piloderma fallax	5.6 (1.4)	10.6 (4.3)	6.4 (3.7)	1.5 (1.2)
Piloderma I	2.0 (0.6)	5.0 (2.0)	11.5 (6.9)	9.5 (2.7)
Piloderma II	1.2 (0.7)	0.7 (0.6)	0	0
Piloderma III	0	0	1.7 (1.7)	3.6 (2.5)
Cortinarius cf *semisanguineus*	1.5 (0.4)	2.4 (1.0)	0.6 (0.5)	0
Cortinarius III	2.2 (1.9)	0	0	0
Cortinarius cinnamomeus	0	1.2 (0.6)	0.9 (0.7)	0
Cortinarius V	0	0.3 (0.3)	0	3.6 (3.6)
Cortinarius hemictrichus	0	0.7 (0.5)	1.1 (0.5)	2.2 (1.6)

Inocybe lanuginosa - like	0	0.5 (0.4)	0.5 (0.3)	1.1 (0.7)
Inocybe I	0	0	0.3 (0.3)	1.5 (1.3)
Leccinum aurantiacum	1.1 (0.7)	0	0	0
Russula decolorans	4.7 (3.9)	1.6 (1.6)	0	0
Russula III	2.2 (2.2)	0	0	0
Russula bicolor	0	0.4 (0.3)	2.2 (0.8)	2.0 (1.5)
Russula I	0	0	1.2 (0.9)	3.0 (2.9)
Russula II	0	0	0	3.7 (2.6)
Thaxterogaster cf *pinguis*	0	5.3 (1.9)	3.8 (1.3)	1.8 (1.0)
Sarcodon sp.	7.9 (3.8)	0	0	0
Tomentella cf *stuposa*	0	0	0	8.1 (2.8)
Unknown fungus I	0.2 (0.2)	2.8 (1.4)	1.7 (0.9)	0
Unknown fungus II	0	0.7 (0.7)	3.0 (2.2)	1.7 (1.7)
Unknown fungus V	0	4.0 (1.7)	5.8 (3.3)	0.4 (0.4)
Unknown fungus VI	0.9 (0.9)	3.4 (1.5)	2.7 (1.6)	0

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

Table 3. Matrix of ectomycorrhizal fungal community similarity (in percent) between plant associations based on species abundance (replicates combined).

P – Cladonia	100			
M – Huckleberry	56	100		
R – Oak fern	42	66	100	
VR – Devil's club	24	35	52	100
	Poor - Cladonia	Medium - Huckleberry	Rich – Oak fern	Very Rich – Devil's club

Fig. 1. ECM species richness per plot (α diversity) in correlation with foliar N concentrations 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 ECM communities among the 19 plots (based on the abundance of 74 species), rotated to maximize correlation with total N. Selected pairwise *p* values: poor vs. medium = 0.003; medium vs. rich = 0.348; medium vs. very rich = 0.006; rich vs. very rich = 0.110.

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

C. geophilum = -245 + 45.2(Foliar $N_{\%}$) – 1.9(Foliar $N_{\%}$)^2; $p = 0.010$, $r^2 = 0.43$;

P. fallax = -191 + 35.4(Foliar $N_{\%}$) – 1.6(Foliar $N_{\%}$)^2; $p = 0.090$, $r^2 = 0.26$;

MRA = 84 - 5.8(Foliar $N_{\%}$); $p = 0.004$, $r^2 = 0.40$;

Unknown fungi VI = 27.6 – 1.9(Foliar $N_{\%}$); $p = 0.00$, $r^2 = 0.45$;

A. byssoides = 0.28 + 0.000014$e^{(\text{Foliar N\%})}$; $p = 0.001$, $r^2 = 0.59$;

L. laccata = 2.1 + 0.0000083$e^{(\text{Foliar N\%})}$; $p = 0.003$, $r^2 = 0.50$

Fig. 4. Exploration types of ectomycorrhizal fungi as a percent of root colonization among plant associations (replicates combined).

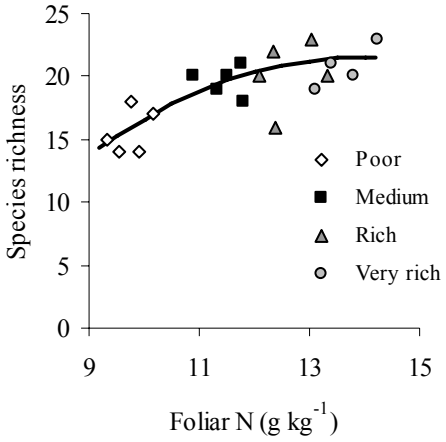
25
20
15
10
5
0
Species richness
9
11
13
15
Foliar N (g kg-1)
Poor
Medium
Rich
Very rich

Fig. 1

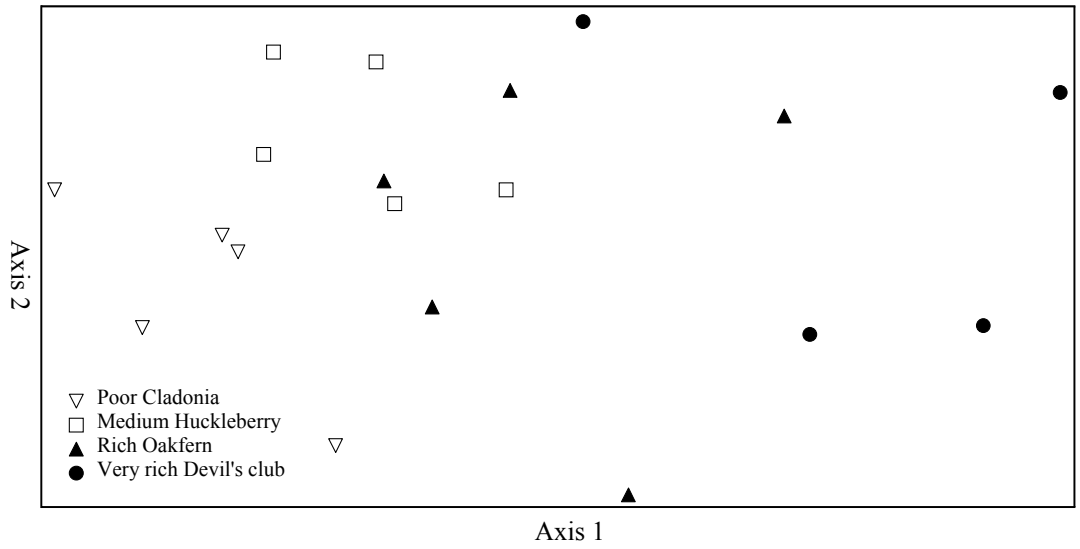
Axis 2
Axis 1
Poor Cladonia
Medium Huckleberry
Rich Oakfern
Very rich Devil's club

Fig. 2

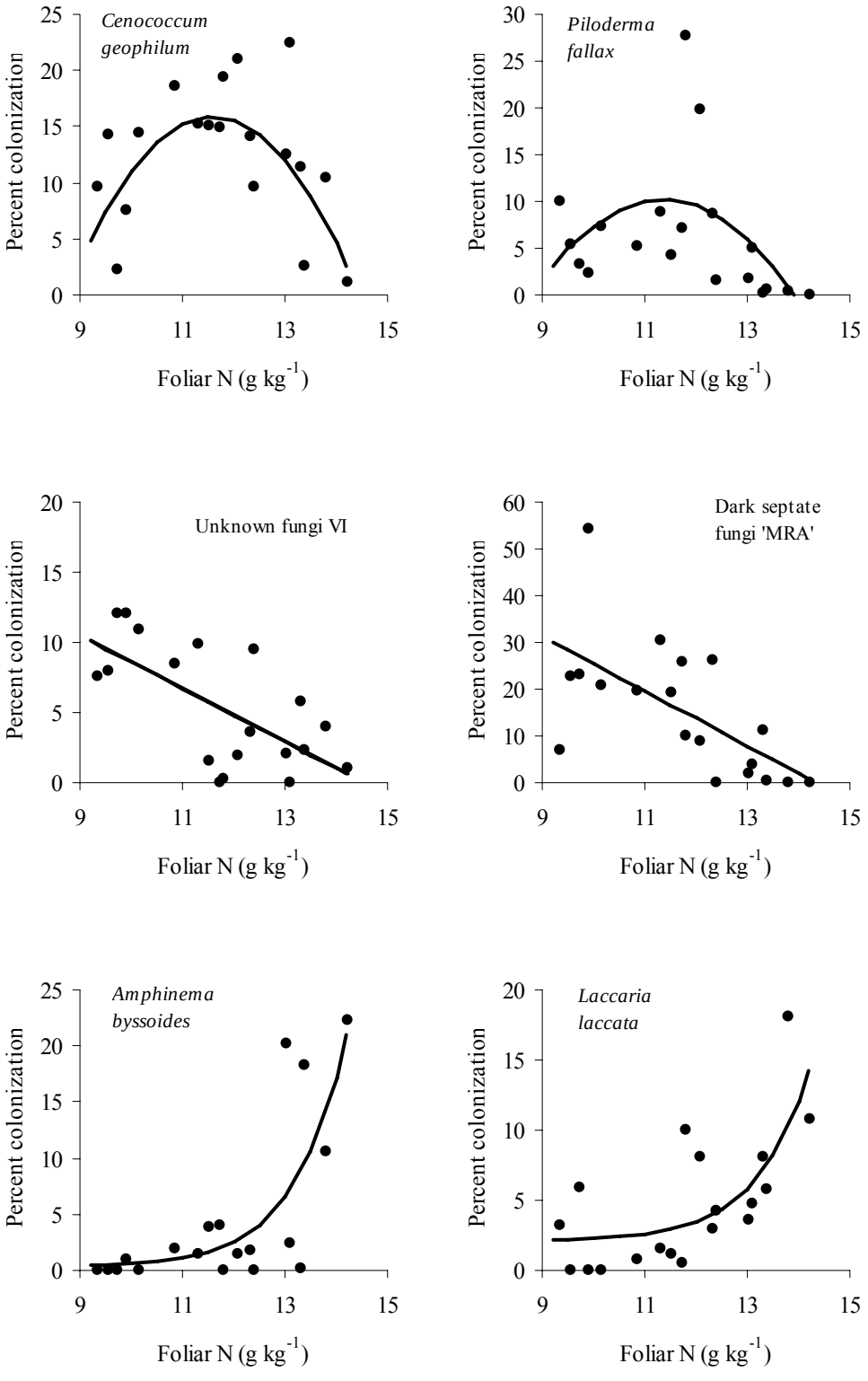
Cenococcum geophilum
Piloderma fallax
Unknown fungi VI
Dark septate fungi 'MRA'
Amphinema byssoides
Laccaria laccata
Percent colonization
Foliar N (g kg-1)

Fig. 3

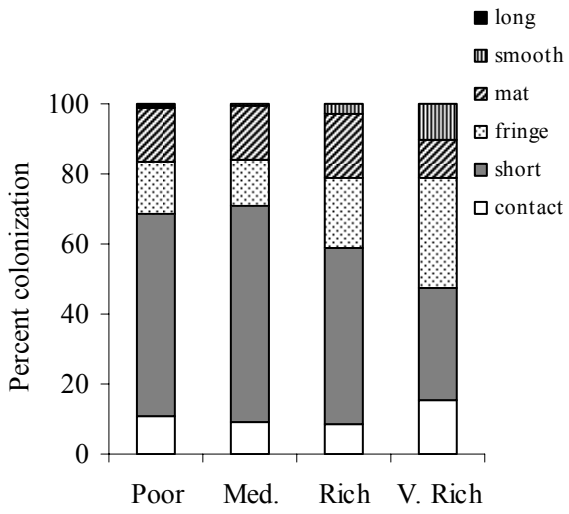
long
smooth
mat
fringe
short
contact
Percent colonization
100
80
60
40
20
0
Poor
Med.
Rich
V. Rich

Fig. 4

Appendix 1. List of morphotypes with successful ITS 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 boulderensis	DQ499466	630/636 (99%)	
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 pluvius	AF389142	630/634 (99%)	
Cortinarius cf *semisanguineus*	DQ481909	580/595 (97%)	Morphotype matched except colour was light pink
Cortinarius I	AY669687	342/348 (98%)	*Cortinarius umbilicatus*
Cortinarius II	AY083191	437/443 (98%)	*Cortinarius humicola*
Cortinarius III	DQ117925	399/399 (100%)	*Cortinarius armeniacus*
Cortinarius IV	AJ889975	370/377 (98%)	*Cortinarius praestigosus*
Cortinarius V	U56030	249/257 (96%)	*Cortinarius paragaudis*
Cortinarius VI	DQ102683	428/446 (95%)	*Cortinarius* cf. *saniosus*
Cortinarius VII	AY669668	476/495 (96%)	*Cortinarius torvus*
Cortinarius VIII	EF218763	511/516 (99%)	uncultured (*Cortinarius*)
Cortinarius IX	DQ365639	586/587 (99%)	Cortinarius sp. SD-113.6

Cortinarius X	AJ438981	578/599 (96%)	*Cortinarius obtusus*
Cortinarius XI	DQ481963	549/552 (99%)	uncultured (*Cortinarius*)
Cortinarius XII	DQ497934	370/387 (95%)	uncultured ectomycorrhiza
Cortinarius XIII	AF325590	487/504 (96%)	*Cortinarius brunneus*
Cortinarius XIV	DQ481959	635/648 (97%)	uncultured cf. Dermocybe
Cortinarius XV	DQ481693	549/552 (99%)	uncultured (*Cortinarius*)
Cortinarius XVI	EF218748	663/673 (98%)	uncultured (*Cortinarius*)
Cortinarius XVII	EF218758	444/446 (99%)	uncultured (*Cortinarius*)
Cortinarius XVIII	AJ534712	486/518 (93%)	Cortinarius sp. O14
Unknown fungi I	EF077497	321/328 (97%)	uncultured ectomycorrhiza
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).