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Influence of soil nutrients on ectomycorrhizal communities in a chronosequence of mixed temperate forests

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Abstract Many factors associated with forests are collectively responsible for controlling ectomycorrhizal (ECM) fungal community structure, including plant species composition, forest structure, stand age, and soil nutrients. The objective of this study was to examine relationships among ECM fungal community measures, local soil nutrients, and stand age along a chronosequence of mixed forest stands that were similar in vegetation composition and site quality. Six combinations of age class (5-, 26-, 65-, and 100-year-old) and stand initiation type (wildfire and clearcut) were replicated on four sites, each representing critical seral stages of stand development in Interior Cedar-Hemlock (ICH) forests of southern British Columbia. We found significant relationships between ECM fungal diversity and both available and organic P; available P was also positively correlated with the abundance of two ECM taxa (*Rhizopogon vinicolor* group and *Cenococcum geophilum*). By contrast, ECM fungal diversity varied unpredictably with total and mineralizable N or C to N ratio. We also found that soil C, N, available P, and forest floor depth did not exhibit strong patterns across stand ages. Overall, ECM fungal community structure was more strongly influenced by stand age than specific soil nutrients, but better correlations with soil nutrients may occur at broader spatial scales covering a wider range of site qualities.

Keywords Soil nitrogen · Soil phosphorus · Mineralizable nitrogen · Douglas-fir · Paper birch · Fungal diversity

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Introduction

Ectomycorrhizal (ECM) fungal communities can be influenced by a variety of factors including ECM tree species composition, forest structure (Villeneuve et al. 1989; Nantel and Neumann 1992; Ishida et al. 2007), stand age (Twieg et al. 2007), and soil nutrients (Avis et al. 2003). Diverse ECM communities can, in turn, have positive effects on host tree productivity (Baxter and Dighton 2001; Jonsson et al. 2001), probably because ECM fungi differ in functional attributes and occupy different environmental niches (Dickie et al. 2002; Courty et al. 2005). While it is clear that realized niches of ECM fungi are determined by host specificity and vigor (Ishida et al. 2007; Simard et al. 2003) as well as by their dispersal and competitive abilities (Peay et al. 2007; Kennedy and Peay 2007), less is known about how they are shaped by their nutrient uptake and stress resistance capabilities, and responses to other environmental factors (Jones et al. 2003). While ECM fungal diversity generally decreases and fungal community composition changes over large gradients of increasing available N (Peter et al. 2001;

Lilleskov et al. 2002; Avis et al. 2003), relationships to soil nutrients are less clear in other cases. For example, in lodgepole pine (*Pinus contorta* var. *latifolia* Doug. Ex Loud) and mixed conifer stands, ECM communities were not correlated with mineral soil nutrients, including available N and P (Douglas et al. 2005). Kernaghan et al. (2003) also found no effects of N or P on ECM communities in boreal mixed forests, but did find reasonable correlations between soil exchangeable cations and abundance of both *Russula* and *Cenococcum* mycorrhizas. While ectomycorrhizas are often concentrated in the fermentation layer of the forest floor (Perez-Moreno and Read 2000), some ECM fungal taxa show preference for mineral horizons (Taylor and Bruns 1999; Dickie et al. 2002; Rosling et al. 2003). This suggests that distribution of ECM fungal species is influenced by nutrient availability, which is generally higher in the fermentation layer than in mineral soils. An alternative explanation, that each ECM fungal species is equivalent in their roles and responses, with differences in distribution being due primarily to dispersal limitations, is provided by neutral theory (Hubbell 2001).

Soil nutrients frequently vary with forest stand age, but in unpredictable ways. Available N and N mineralization can be higher in young stands than in older stands (Prescott 1997; Thibadeau et al. 2000; Bradley et al. 2002), but this is not always the case (Barg and Edmonds 1999; Griffiths and Swanson 2001; Kranabetter and Coates 2004). Organic phosphorus can also be more abundant in recently disturbed than mature stands (Qualls et al. 2000), or may not differ (Kranabetter and Coates 2004). The C to N ratio of the forest floor can decrease after clearcutting (Olsson et al. 1996), which is likely to affect nutrient cycling because the microbial community may become carbon limited (Prescott 2002). Although models of total organic matter in forest floors often predict losses from the time of forest harvest up to about 20 years of age, the presence and extent of this loss varies substantially among studies (reviewed in Yanai et al. 2003).

In a previous paper, we described how the species composition of the ECM fungal community changed along a chronosequence of mixed forest stands that were similar in vegetation composition and site quality (Twieg et al. 2007). In this paper, we describe how local soil nutrients influence ECM fungal community measures. Stand age strongly affects ECM communities on these and other sites (Visser 1995; Smith et al. 2002; Twieg et al. 2007); however, stand age did not account for all of the variation. The current study addresses whether variations in soil nutrients can account for the remaining variation not accounted for by the age of the sites. Our null hypothesis is that soil nutrients have no effect on ECM fungal communities, following neutral theory. We test the following more specific, alternative hypotheses: (1) soil nutrients explain a significant degree of variation in ECM diversity that is not accounted for by stand age; (2) soil nutrients are related to abundance of ECM taxa, and hence ECM community composition; (3) soil N and P availability and mineralizable N decrease while C to N ratio increases with stand age; and (4) stands that are more similar in tree community composition are also more similar in ECM community composition. In order to test for evidence of different functional attributes among the most abundant ECM, we tested them for phosphomonoesterase activity. The presence of different enzyme activities among ECM associated with soils differing in nutrient status would provide evidence for niche partitioning among the ECM fungi.

Materials and methods

Site description and experimental design

Six combinations of age class and stand initiation type (Table 1) were selected to represent critical seral stand development stages in the Shuswap Moist Warm (ICHmw2), Thompson Moist Warm (ICHmw3), and Thompson Moist Cool (ICHmk1) biogeoclimatic variants of the Interior Cedar-Hemlock (ICH) forests of southern British Columbia (Lloyd et al. (1990); see Twieg et al. 2007). These variants are distinguished by their precipitation and temperature regimes, with annual averages of 656 mm and 7.5°C in the ICHmw2, 671 mm and 5.3°C in the ICHmw3, and 665 and 2.8°C in the ICHmk1 (Lloyd et al. 1990). Wildfire-origin (burned) sites in 5-, 26-, 65-, and 100-year-old age classes, represented stand initiation, stem exclusion, stand re-initiation, and mature seral stages of stand development, whereas clearcut-origin sites were only in 5- and 26-year-old age classes. We refer to each unique combination of stand age and initiation type as a "stand type"; for example, 26-year-old burned stands and 26-year-old clearcuts are two different stand types. There were four replicate sites for each stand type, for a total of 24 sites. All sites were dominated by Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and paper birch (*Betula papyrifera* Marsh.). Two of the 26-year-old burn sites were classified as ICHmk1; all others were in the ICHmw variants. Site attributes are detailed in Twieg et al. (2007). Total crown cover, and cover of tree species or groups, were estimated along four parallel transects (two 5-m radius plots each) perpendicular to the aspect, spaced approx. 5–6 m apart. Canopy cover was estimated visually at two systematically located points along each transect: (1) Douglas-fir; (2) paper birch; (3) other ECM conifer hosts (hybrid spruce (*Picea engelmannii* Engelm x *Picea glauca* (Moench) Voss), western hemlock (*Tsuga hetero-*

Table 1 Tree cover variables by site

Site	Stand type	Actual age	Percent canopy cover					% Understorey cover—other ECM conifers	% Understorey cover—Western redcedar
			DF	PB	Other ECM conifers	Other ECM broadleaves	Western redcedar		
19MR	5-year-old cc	6	5	33	8	1	4	0	0
AL	5-year-old cc	6	11	23	8	3	0	0	0
BC	5-year-old cc	4	3	55	3	3	0	0	0
WL	5-year-old cc	5	5	35	9	1	1	0	0
ED1	26-year-old b	30	28	32	6	10	0	8	8
ED2	26-year-old b	30	30	38	9	4	0	3	3
MA1	26-year-old b	24	28	32	4	7	0	13	8
MA2	26-year-old b	24	32	28	5	5	0	8	3
DISC	26-year-old cc	27	26	39	0	0	0	0	0
NM	26-year-old cc	21	24	27	0	3	6	13	8
SRC	26-year-old cc	22	36	38	0	2	0	0	3
ZP	26-year-old cc	25	28	28	7	7	0	8	3
BA	65-year-old b	63	38	43	4	0	4	0	3
MARA	65-year-old b	71	36	40	0	0	4	3	8
RR	65-year-old b	61	30	45	2.5	0	0	8	0
SL	65-year-old b	68	41	41	5	2	2	0	8
4WD	100-year-old b	103	45	41	0	0	5	0	0
ACR	100-year-old b	98	44	24	6	0	6	0	13
BBP	100-year-old b	101	32	32	5	0	8	8	3
WAP	100-year-old b	90	40	32	0	0	8	3	8

DF Douglas-fir, *PB* paper birch, *cc* clearcut, *b* burned for stand type

phylla (Raf.) Sarg.) (canopy in 5-year-old stands only; understorey in older stands), western white pine (*Pinus monticola* Dougl. Ex D. Don in Lamb.), and lodgepole pine); (4) other ECM broadleaf hosts (black cottonwood (*Populus trichocarpa* Torr. & Gray), trembling aspen (*Populus tremuloides* Michx.), and willow (*Salix*) spp.); and (5) western redcedar (*Thuja plicata* (Donn ex D. Don) Spach). Percent cover of understorey ECM conifers and western redcedar was also estimated. Site index for each site was based on the forest cover inventory determined using *SIBEC* standards (British Columbia Ministry of Forests and Range 2007).

Sampling methods

Complete methods for sampling and characterizing the below-ground ECM fungal communities of Douglas-fir and paper birch are presented in Twieg et al. (2007). Briefly, ECM fungal symbionts were identified from 100 ECM root tips in each of ten soil samples collected from a 30×30 m area on each site (1,000 root tips per site), half of which were sampled in spring and the other half in fall, 2004. Each soil sample consisted of a 9×9 cm square core and included the forest floor and top 20 cm of mineral soil, which were bagged and processed separately. If sufficient numbers of ECM root tips were available in both the mineral soil and forest floor of each sample, then 50 tips from each layer were examined; if one layer did not have at least 50 tips, then more tips were sampled from the other layer to bring the total ECM tips examined per soil sample to 100. Even numbers of ECM tips were sampled from Douglas-fir and paper birch hosts in each site. For the 5-year-old burned sites, ectomycorrhizas were examined from excavated Douglas-fir seedlings because soil samples yielded insufficient root tips. Due to this sampling difference, analyses of ECM fungal communities exclude the 5-year-old burned sites. ECM communities of Douglas-fir seedlings from 5-year-old burned and clearcut sites are compared in Twieg et al. (2007). Fungal identification was based on a combination of detailed morphology and anatomy, observed under both stereo and compound microscopes (Goodman et al. 1996) as well as sequencing of the internal transcribed spacer (ITS) region of rDNA (Twieg et al. 2007).

Ectomycorrhizas of the most common types were selected from a random subset of cores for the assay of phosphomonoesterase. Seven tips per mycorrhizal type per

core were assayed within 24 h of being removed from soil. Activities were quantified by measuring fluorescence of products released from 4-methylumbelliferone (MU)-labeled substrates, according to the microplate method developed by Pritsch et al. (2004). After the assay, the projected area of each tip was calculated using WinRhizo™ (Regent, Québec) and the activities expressed as pg MU per square millimeter per minute.

Soil samples for chemical analysis were collected in August 2004 from all 24 sites. The sampling period was chosen because it represents a consistently dry period, whereas at other times rainfall is unpredictable temporally and spatially. This sampling strategy allowed us to compare sites without interference from short-term effects of soil moisture on processes such as nitrogen mineralization. Approximately 1 kg of mineral soil and 300 g of forest floor were removed from eight locations per site. These were immediately adjacent to where ectomycorrhizal tips were sampled; four were placed next to the spring samplings and four next to the fall samplings. At the 5-year-old wildfire sites only, soils were sampled next to eight randomly selected Douglas-fir seedlings located within 3 m of a paper birch tree. No forest floor samples were taken from these burned sites due to the near absence of forest floor layers. Mineral and forest floor samples were bulked separately for each site, separated into three subsamples, sealed in polyethylene bags, and transported on ice in coolers to the lab. Forest floor thickness was measured at each soil sampling location during ECM sampling in spring and fall, yielding 16 observations per site.

Mineral soil samples were sieved to 2 mm, air-dried, and sent to the BC Ministry of Forests and Range Analytical Chemistry Laboratory in Victoria, BC for all soil analyses except organic phosphorus. Forest floor samples were air-dried, passed through a 4-mm sieve, and milled prior to analysis. Total C and N were determined by combustion elemental analysis, using a Leco CHN-600 Elemental Analyser (Leco Corp., St. Joseph, MI, USA). Available ammonium-N and nitrate-N were extracted by shaking for 2 h in 2-N KCl (Bremner 1996) and their concentrations measured on a Technicon AutoAnalyser II. Potentially mineralizable N was estimated by anaerobic incubation (Waring and Bremner 1964) in waterlogged conditions at 30°C for 2 weeks, followed by extraction and analysis for ammonium and nitrate as above. Initial available N values were subtracted from post-incubation values to estimate mineralizable N. We were unable to measure in situ N availability, but earlier studies in nearby forests of the same composition showed that mineralizable N was correlated with N availability measured using in situ buried bag incubations (Forge and Simard 2000). Available P was determined using the Bray-1 method. Organic phosphorus of the forest floor was estimated from the difference in sulfuric acid-extractable phosphorus between post-ignited and pre-ignited soil samples, as described in Olson and Sommers (1982). Measurements were averaged from five readings on the spectrophotometer for each sample.

Data analysis

Statistical analyses were carried out in SAS v. 9.1 (SAS Institute, Carey, NY, USA) and significance for all statistical tests was set at α=0.05 unless otherwise noted. In order to test hypothesis 3, one-way analysis of variance (ANOVA) was used to test for differences among stand types for all soil variables (see Table 2 for complete list of variables), using averages of the three subsamples per site. Stand type means were separated using Bonferroni multiple comparison tests. Where data violated assumptions of normality and equal variance, they were transformed by the natural log and assumptions were re-examined.

Evenness values of ECM fungal communities were calculated in PC-Ord v. 4 (McCune and Mefford 1999) for forest floor and mineral soil layers separately, and at both individual soil sample and site levels. Paired *t* tests were used to compare site-level species richness and evenness between the forest floor and mineral soil. Generalized linear models, based on maximum likelihood and the Poisson distribution, were used to predict ECM species richness per soil sample. All possible combinations of the following predictor variables were used: stand age, 1/stand age, number of tips identified from the forest floor and mineral soil (separately), and forest floor thickness. Akaike's Information Criterion (AIC) was used to select the best model from the resulting set of candidate models (Burnham and Anderson 2002).

Relative abundances of the most abundant taxa were calculated for mineral and forest floor layers by dividing the total number of tips of each taxon in each layer by the total number of ECM tips examined from each site on the appropriate host(s) (e.g., if a fungal species was found only on Douglas-fir, then the relative abundance of that species was calculated relative only to the total number of Douglas-fir ectomycorrhizas examined, but fungal species found on both hosts were evaluated compared to the total number of ectomycorrhizas identified from both hosts) . Two-tailed paired *t* tests were used to compare relative abundance of taxa between layers. Sites without the concerned taxon were removed from this analysis because a lack of differences between soil layers is less informative in these cases.

Stepwise least-squares multiple regression was used to explore relationships between ECM diversity and soil nutrients. To do this, site scores for the first Principal Component of a Principal Components Analysis (PCA) on species richness, Shannon's diversity index, Simpson's diversity, evenness, and first-order jackknife estimates for each of: combined, Douglas-fir, and paper birch communi-

Table 2 Mean soil nutrients by stand age and initiation type (n=4; standard error of mean in parentheses)

Stand age and initiation type	C/N ratio	Initial ammonium	Initial nitrate	Mineralizable ammonium	Mineralizable nitrate	Organic P	Available P
Mineral soil							
5-year-old burned	23.2 (0.55) a	2.60 (0.42)	0.18 (0.06)[a]	18.3 (1.3)	0.678 (0.149) ab	N/A	143 (24)
5-year-old clearcut	29.9 (1.48) ab	1.97 (0.18)	0.33 (0.03)	15.3 (1.8)	0.730 (0.145) ab	N/A	159 (19)
26-year-old burned	29.6 (1.94) ab	2.23 (0.51)	0.38 (0.06)	14.4 (2.8)	0.974 (0.128) b	N/A	208 (49)
26-year-old clearcut	35.0 (1.13) b	2.44 (0.38)	0.28 (0.04)	9.2 (3.2)	0.971 (0.111) b	N/A	95 (16)
65-year-old burned	24.6 (0.56) a	2.18 (0.33)	0.38 (0.02)	14.8 (2.7)	0.490 (0.114) a	N/A	139 (18)
100 year-old burned	25.3 (0.79) ab	2.02 (0.27)	0.30 (0.02)	14.1 (1.3)	0.442 (0.128) a	N/A	209 (24)
F ratio	4.11	0.45	3.00	1.68	5.28	N/A	0.88
P value	0.0115	0.8108	0.0386	0.1914	0.0037	N/A	0.5162
Forest floor							
5-year-old clearcut	47.0 (2.55) b	11.7 (2.5)[a]	0.39 (0.23)	230 (29)	1.111 (0.193)	685 (121)	101 (11)
26-year-old clearcut	38.3 (1.17) ab	27.1 (3.5)	0.95 (0.22)	375 (22)	1.570 (0.344)	973 (108)	133 (16)
26-year-old burned	34.1 (1.80) ab	22.8 (2.3)	0.58 (0.14)	440 (34)	1.660 (0.310)	980 (39)	150 (7.4)
65-year-old burned	35.2 (1.58) ab	18.4 (3.8)	0.83 (0.34)	309 (29)	1.512 (0.246)	765 (55)	112 (8.6)
100-year-old burned	33.7 (1.05) a	26.9 (4.8)	1.0 (0.39)	411 (48)	1.363 (0.219)	954 (63)	102 (6.2)
F ratio	3.09	3.40	0.93	1.94	0.54	2.67	1.24
P value	0.0485	0.0362	0.4717	0.1557	0.7807	0.0729	0.3357

Values expressed as mg kg^{-1} soil except C/N ratio. Means followed by the same letter (within one soil layer and variable) are not significantly different (p>0.05) by multiple comparisons. F ratios and p values are from one-way ANOVAs

[a] Difference among treatment means, but no significant differences found in pairwise mean comparisons

ties were predicted (see Twieg 2006). Species richness in the combined community (i.e., both hosts), and species richness on Douglas-fir were also predicted from the following variables for mineral soil and forest floor: C to N ratio, available N, potentially mineralizable N, and available P. Stand age, forest floor organic P, site index, and biogeoclimatic variant were also used as predictor variables. Biogeoclimatic variant was included only to control for variability introduced by having two sites from a moist-cool variant as opposed to moist-warm variants, which had been found to affect ECM fungal communities in Twieg et al. (2007). The criteria for entry and retention in the models were α=0.15 for model F test and α=0.1 for predictor partial F test significance.

Each tree cover variable had 7–10 zero values out of the 20 sites, rendering these data inappropriate for multiple regression analyses. The ranges of values in tree cover variables were also fairly low (see Table 1) because the sites were intentionally selected to be relatively pure mixtures of Douglas-fir and paper birch. A Mantel test was therefore used to determine whether site similarity in the ECM community was related to site similarity in tree cover variables. Frequency of ECM fungal species from the combined community of both host species was used in one matrix, and the tree cover variables presented in Table 1 were used in the other matrix. The Relative Sorensen distance measure was chosen for the ECM community matrix and Sorensen distance for tree cover, and tests based on both Monte Carlo randomizations (1,000) and Mantel's Asymptotic z Approximation were checked for significance of the intermatrix correlation. This analysis was performed both with and without 5-year-old sites included because root systems of other tree species in the young stands were much less likely to overlap those of target hosts and were therefore less likely to affect their ECM fungal communities.

Stepwise regressions were used to predict relative abundance of *Rhizopogon vinicolor* type (Douglas-fir only), and *Cenococcum geophilum* and the genus *Russula* in the combined community. These taxa were chosen because of their high frequency and abundance, and because their relative abundance data met normality and homoscedasticity assumptions. Canonical Correspondence Analysis (CCA) was used in PC-ORD to ordinate sites and ECM fungal species based on species frequencies (the number of soil samples per site in which each species occurred) in the combined community of both hosts. The same soil variables used in regressions on diversity variables were used as the environmental matrix. Only species that occurred in more than two sites were included because the Chi-square distance measure implicit in CCA can give undue weight to rare species (McCune et al. 2002). Monte Carlo randomizations (1,000) were used to test significance

of site ordination and correlation between the environmental variables and ECM communities. Stepwise regression analysis was also used with stand age and soil variables as predictors for sites' axis scores from a well-structured NMS ordination of the entire ECM fungal community (see Twieg et al. 2007).

Results

Root tip number

The total number of root tips included in site-level analyses was similar for all sites (see Twieg et al. 2007), but the average number of root tips examined from the forest floor was about 30% higher than the number examined from mineral soil. More mineral soil samples had fewer than 50 ECM root tips available than did forest floor samples (141 vs. 99, respectively). The ratio of the number of root tips examined from the forest floor layer to mineral soil varied considerably by site, but did not differ among stand types (F=1.9; p=0.17); nor was it useful in predicting ECM fungal diversity across sites (p=0.33).

ECM community richness and diversity

Details of fungal diversity by stand type and species-sample unit curves can be found in Twieg et al. (2007). The cumulative number of ECM fungal species detected (i.e., from all sites pooled) was similar in the mineral soil and forest floor layers (73 and 82, respectively). Mean site-level species richness, however, was 27% higher in the forest floor (13.6±0.91 SEM) than the mineral soil (10.8±0.77 SEM) (t=−3.02; p=0.0007). Site-level evenness of the ECM community did not differ between soil layers (t=−0.16; p=0.87). According to AIC, the best models predicting per soil sample ECM fungal richness of the combined community of both hosts included the number of root tips examined from the forest floor, as well as stand age, the former of which varied because many samples lacked sufficient root tips in either the forest floor or mineral soil to provide an equal number from each layer per sample. Neither the number of tips from the mineral soil nor forest floor thickness was a significant predictor of ECM fungal richness of both hosts combined. Likelihood tests were also significant for the number of tips examined from the forest floor (χ^2=4.09; p=0.0432) as well as for stand age (χ^2=6.20; p=0.0128). Including the forest floor root tip number increased soil sample-level prediction of ECM species richness on Douglas-fir and the combined community by about one species per 10 root tips. Regression models predicting site-level ECM richness and diversity are summarized in Table 3. In addition to stand age, biogeoclimatic variant was also a significant predictor of ECM species richness and diversity in some cases, possibly because of the tendency for lower hemlock abundance on the two ICHmk1 sites. Forest floor organic P was positively related to ECM diversity PCA axis one scores and ECM diversity on Douglas-fir. Mineral soil available P was negatively related to ECM fungal richness on Douglas-fir.

Table 3 Summary of stepwise regression analyses predicting ECM diversity variables

Y variable/*x* variables	Range of *Y* variable	Range of *X* variable	Slope or intercept estimate	Partial *F* test *P* value	R^2	Model *F* test *P* value
Diversity principal component	−6.92 to 4.35					0.0001
1/stand age		0.009 to 0.25	−27.407	0.0004	0.51	
Biogeoclimatic variant		0 (ICHmw); 1 (ICHmk)	−4.148	0.0074	0.65	
Forest floor organic P		412 to 1,166	0.0043	0.0590	*0.72*	
Intercept		N/A	2.054	0.3703		
Richness of combined community	9 to 27					0.0010
1/stand age		See above	−46.397	0.0006	0.40	
Biogeoclimatic variant		See above	−6.44	0.0246	*0.56*	
Intercept		N/A	21.991	<0.0001		
Richness of Douglas-fir community	2 to 16					0.0001
Stand age		4 to 103	0.0885	<0.0001	0.52	
Forest floor organic P		See above	0.00771	0.0177	0.62	
Mineral soil available P		58 to 484	−0.0131	0.0294	*0.72*	
Intercept		N/A	0.9157	0.7279		

R^2 values in italics correspond to the model that contains all significant predictor variables; those not in italics correspond to the model including that predictor variable and those above it for the corresponding dependent variable

Major ectomycorrhizas and community composition

Several frequently observed ECM taxa, through an analysis at the site level which excluded stand age, occurred in the forest floor and mineral soil, but showed preference for one layer. *Leccinum scabrum*, *Rhizopogon rudus*, and *Suillus lakei* were more abundant in the mineral soil, while *Lactarius torminosus* and the genera *Cortinarius*, *Hebeloma*, and *Piloderma* were more abundant in the forest floor (Table 4). *C. geophilum*, *Lactarius pubescens*, *Lactarius scrobiculatus*, *R. vinicolor* group and the genus *Russula* did not differ in relative abundance between soil layers. *R. vinicolor* group includes two recently separated species, *R. vinicolor* and *Rhizopogon vesiculosus* (Kretzer et al. 2003); limited resources did not permit DNA analysis of all samples of these two species, which are not reliably separated by morphology. Fifty-five of the 105 identified species of ECM fungi were found in only one of the two soil layers, but these species were too infrequent to evaluate their soil layer preference.

There were also significant relationships between some soil nutrient variables and the relative abundances of dominant ECM taxa. Available N and P in mineral soil and available P in the forest floor were significant in models predicting *C. geophilum* relative abundance, and available P in mineral soil and forest floor were significant in predicting *R. vinicolor* group relative abundance (Table 5). Out of the variables that were significant in predicting *C. geophilum* and *R. vinicolor* group relative abundances from both soil layers combined, only available P in the forest floor was significant in regressions predicting abundances from each soil layer separately (data not shown). Mycorrhizas formed by *R. vinicolor* group, *L. torminosus*, and an unknown *Russula* species (*Russula* 9) were common enough in the selected samples to analyze statistically for enzyme activity. Phosphatase activities differed among mycorrhizas (F=3.81; p=0.043), with activities on the surface of *R. vinicolor* group mycorrhizas significantly lower (14±3 pg MU $mm^{-2}min^{-1}$) than on *L. torminosus* mycorrhizas (27±3 pg MU mm^{-2} min^{-1}), and with the *Russula* mycorrhizas intermediate (17± 3 pg MU $mm^{-2}min^{-1}$) in activity.

Tree cover variables were weakly correlated with ECM community. The relationship was stronger with 5-year-old sites included (Mantel's Standardized r=0.44; p=0.00001 and 0.001 for Mantel's Asymptotic Approximation and Monte Carlo tests, respectively) than when they were removed (r=0.25; p=0.016 and 0.007). Roughly half of the ECM fungal species occurred in two or fewer sites, and were removed for CCA. In addition to stand age (r=−0.919 to axis 1), three soil or site productivity variables were moderately correlated to one axis each in the CCA ordination when tested individually (Fig. 1): site index (r=−0.558 to axis 3); mineral soil C to N ratio (r=0.533 to axis 1); and mineral soil available phosphorus (r=0.523 to axis 2). Overall correlations between species and environmental matrices were not significant (p=0.057, 0.515, and 0.0243 for axes 1–3, respectively). The 0.057 p value for correlation between axis one and the environmental matrix is likely because that axis one has a very strong relationship to stand age and rather weak relationships to most other variables. No soil variables were significant in predicting axis scores from a well-structured NMS ordination of the combined community, nor was there a significant correlation between the ECM community and soil variable matrices (Mantel's Standardized r=0.10; p values=0.32 and 0.18 for Mantel's Asymptotic Approximation and Monte Carlo tests, respectively).

Table 4 Mean relative abundances (on roots examined from the designated host) of dominant taxa by soil layer

ECM taxon	Host tree	Number of sites (n)	Mineral soil relative abundance (%)	Forest floor relative abundance (%)	P value: paired T test
Rhizopogon vinicolor type	DF	20	18.0	23.5	0.266
Rhizopogon rudus	DF	6	8.95	2.39	*0.026*
Suillus lakei	DF	9	14.9	2.86	*0.016*
Lactarius pubescens	PB	5	8.84	7.48	0.759
Lactarius torminosus	PB	10	2.74	12.1	*0.033*
Leccinum scabrum	PB	16	8.70	1.58	*0.012*
Cenococcum geophilum	Both	20	4.11	6.15	0.059
Cortinarius spp.	Both	16	0.91	4.40	*0.030*
Hebeloma spp.	Both	11	1.43	4.79	*0.003*
Lactarius scrobiculatus	Both	11	1.96	4.24	0.245
Piloderma spp.	Both	14	0.89	7.68	*0.002*
Russula spp.	Both	18	7.08	6.66	0.881

Significant differences have p values in italics (paired t test)

DF Douglas-fir, *PB* paper birch

Table 5 Summary of stepwise regression models predicting relative abundances (%) of dominant ECM taxa

Y variable/*x* variables	Range of *Y* variable	Range of *X* variable	Slope or intercept estimate	Partial *F* test *P* value	R^2	Model *F* test *P* value
Cenococcum geophilum	2.9 to 18.8%					0.0005
Mineral soil available N		1.55 to 3.94	4.383	0.0005	0.41	
Forest floor available P		45 to 195	0.0563	0.0100	0.51	
Mineral soil available P		58 to 484	−0.0200	0.0152	*0.66*	
Intercept		N/A	−4.367	0.1696		
Rhizopogon vinicolor type	5.3 to 89.1%					<0.0001
1/stand age		0.009 to 0.25	295.11	<0.0001	0.64	
Forest floor available P		See above	0.1570	0.0695	0.74	
Mineral soil available P		See above	0.0568	0.0770	*0.79*	
Intercept		N/A	−4.09	0.6898		
Russula spp.	0 to 37.8%					<0.0001
Stand age		See above	0.2749	<0.0001	*0.68*	
Intercept		N/A	0.6974	0.7787		

R^2 values in italics correspond to the model that contains all significant predictor variables; those not in italics correspond to the model including that predictor variable and those above it for the corresponding dependent variable

Soil characteristics across stand types

The C to N ratio of mineral soil was highest in the 26-year-old clearcut stands and that of forest floor was 40% higher in 5-year-old clearcut stands than 100-year-old stands (Table 2). Total C content of the forest floor was similar among stand types (range 34.4–38.5%), but mineral soil total C was higher in 26-year-old clearcuts than 65-year-old burned sites, with all other treatments intermediate in total C (F=3.02, p=0.037, data not shown). There were no significant differences among stand types in total N, although the lower C to N ratio of the forest floor in 100-year-old stands was primarily due to slightly lower total N in the 5-year-old clearcuts (data not shown). They did differ in available nitrate-N of the mineral soil and available ammonium-N of the forest floor, but multiple comparison tests revealed no pairwise differences. As expected, mineral soil mineralizable nitrate-N averaged only 5% of mineralizable ammonium-N levels (range 3.1–10.5%), and were twice as high in 26-year-old sites than 65- and 100-year-old sites. Stand types did not differ significantly in any of the other soil parameters measured. Forest floor thickness did not differ among stand types (average=5.15 cm; F=1.26; p=0.29).

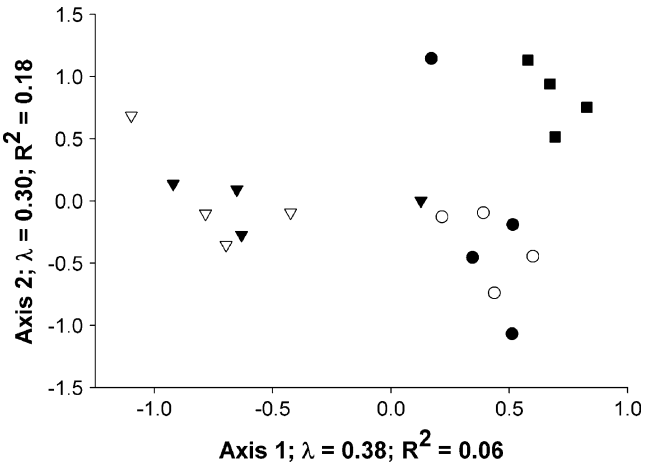

Fig. 1 CCA ordination of sites based on frequency of ECM fungi in the combined community of both hosts and environmental variables (stand age, site index, and soil variables). *Squares*, 5-year-old clearcut; *filled circles*, 26-year-old burned sites; *open circles*, 26 year-old clearcut sites; *filled triangles*, 65-year-old burned sites; and *open triangles*, 100-year-old burned sites. λ, axis eigenvalue; R^2, proportion of variance in Chi-squared distance among sites explained by ordination axes

Discussion

There was considerable variation in soil nutrients among the sites, even though all were mesic sites (Lloyd et al. 1990). In accordance with our first alternative hypothesis, one soil variable, available P, was negatively related to fungal species richness on Douglas-fir. The relative abundance of *R. vinicolor* group mycorrhizas was also strongly negatively related to fungal species richness on Douglas-fir (see Twieg et al. 2007), and the relative abundance of these mycorrhizas was positively related to available P, partially supporting our second alternative hypothesis that soil nutrients are related to abundance of particular ECM taxa. Since available P was not related to

diversity measurements that included the paper birch ECM fungal community, it is possible that this negative relationship between available P and ECM fungal richness on Douglas-fir is due in part to the relationship between available P and *Rhizopogon* abundance. We have previously found that *Rhizopogon* can dominate root systems of Douglas-fir seedlings in a similar ecosystem, effectively reducing ECM fungal diversity (Jones et al. 1997). The observation that *R. vinicolor* group mycorrhizas were associated with lower phosphatase activities suggests that these fungi may be better adapted to soils with higher available inorganic P. The abundance of *C. geophilum* in the combined community of both hosts was also positively related to forest floor available P. Hence, greater available P seems to be an important driver of ECM community structure in these forests, possibly by influencing competitive interactions among ECM fungi. Available P has also been found to be associated with ECM species distributions in other ecosystems (Morris et al. 2008).

It is somewhat surprising that soil N and C to N ratio were not stronger predictors of the ECM community, given the results of earlier studies; however, significant N effects in these earlier studies may have been due to stronger N gradients. For example, Lilleskov et al. (2002) found that below-ground ECM diversity was negatively related to extractable (available) mineral N in forest floors in the Kenai Peninsula of Alaska; however, the highest values of N in their study were extreme compared to our study. Furthermore, Avis et al. (2003) and Peter et al. (2001) both found that N addition affected species richness and diversity of the ECM fungal sporocarp communities more than root tip communities, possibly because of a change in resource allocation (Lilleskov and Bruns 2001). In our case, we did find a relationship between available N of the mineral soil and relative abundance of *Cenococcum* in the combined mineral soil and forest floor layers. Although below-ground *Russula* abundance has been shown to decrease (Peter et al. 2001) or increase (Avis et al. 2003) following N fertilization, this genus was not related to N at the levels observed in our study.

Although total ECM root tips in the mineral soil and forest floor were not directly counted, they appeared more densely and regularly distributed in the forest floor than mineral soil. The overall preference of ectomycorrhizas for the forest floor over the mineral soil was particularly apparent because a much higher volume of mineral soil than forest floor was examined on average per sample, and yet there were many more mineral soil samples than forest floor samples that contained fewer than fifty ECM root tips. ECM tips have previously been shown to be concentrated more in the forest floor than mineral soil (Erland and Taylor 2002; Nelville et al. 2002), which probably reflects greater resource availability and heterogeneity in the forest floor. Site-level and soil sample-level ECM fungal richness were significantly, but not substantially, higher in the forest floor than the mineral soil. However, high cumulative ECM fungal diversity and preference of some ECM fungal species for mineral soil suggests that both forest floor and mineral soil require sampling to accurately characterize ECM communities.

Although we found significant effects of some soil nutrient variables on ECM fungal community composition when considered individually, canonical correspondence analysis of sites showed little overall correlation between ECM fungal communities and environmental variables. It was somewhat surprising that C to N ratio was correlated with CCA axis one in the opposite direction as stand age, but the lack of strength of this correlation and somewhat low statistical significance of the overall correlation between fungal and environmental matrices on this axis prevents sound speculation on this relationship. NMS ordination (see Twieg et al. 2007) accounted for much more variation in ECM community structure than did CCA. This may be attributed partly to the fact that the implicit Chi-square distance measure in CCA gives higher weight to rare species than the Relative Sorensen distance measure that was applied in NMS (McCune et al. 2002), but also indicates that the measured soil variables were not related to ECM communities in predictable ways.

The correlation between ECM fungal community and tree cover variable matrices was fair. When the 5-year-old sites were removed from the analysis, the correlation was weakened, suggesting that this age class may have had a disproportionate effect on the correlation structure of fungal communities and community structure of tree species. Ordinations indicate that the ECM fungal communities in 5-year-old sites were similar to each other, which corresponded with low canopy cover of Douglas-fir. Cover of other ECM conifers was nearly equal to that of Douglas-fir in these young stands because of the recent practice of planting mixtures of several conifer species that occur naturally in the study area after clearcutting. It is therefore difficult to ascertain whether or not other ECM tree species were affecting ECM fungal communities of Douglas-fir and paper birch in the young stands, even though there appeared to be limited below-ground interaction with other conifer species. Spatial analysis of ECM fungal communities in these stands would help determine the importance of tree mixtures in these young stands. Overall, this study does not provide strong evidence that relatively minor variations in tree community composition can explain a high proportion of variation in ECM community variation that is not explained by stand age. Even so, the unique ECM fungal community and soil nutrient availability of the 5-year-old stands supports concepts in forest ecology that post-disturbance conditions are more extreme, dynamic,

and important than other stand developmental stages in setting the trajectory of future ecosystem succession patterns (Chapin et al. 2002).

The data did not support the third alternative hypothesis that mineral forms of N and P are more available, and C to N ratio lower, in younger stands. Indeed, no pattern in C, P, or forest floor depth across stand ages was evident, and in particular, no pattern that paralleled the strong increase in ECM fungal diversity seen from 5-year-old to 26-year-old age classes. Although mineralizable N is commonly higher in recent clearcuts or forest gaps than in mature forests, this effect is most pronounced in the first year following disturbance and then subsides (Prescott 2002; Bauhus and Barthel 1995). In this study, an early N flush would have been missed because the youngest age class sampled was 5 years old. Consistent with this, (Kranabetter and Coates 2004) found no difference in soil available N and P, or organic P between mature ICH stands and 10-year-old plantations. Forge and Simard (2000) found that mineralizable N was lower in 10 year-old plantations than adjacent mature ICH forests, probably because of uptake by the lush herbaceous vegetation layer that had developed after harvest. This result is consistent with the results in our study; both available and mineralizable N in the forest floor was lower in 5-year-old stands than older stands.

Conclusions

Although we found that available P explained a significant amount of the variation in ECM fungal diversity not explained by stand age, overall ECM community structure was not strongly related to soil nutrients at the site level in this study. This supports our null hypothesis that there was little spatial niche partitioning among fungal species. Two exceptions were *R. vinicolor* type and *C. geophilum* mycorrhizas on Douglas-fir; their abundances were positively related to soil available P, which, in turn, was negatively related to Douglas-fir fungal species richness, providing insight into the relative competitive abilities of these specific fungi for phosphorus. For the other taxa, there appears to be considerable functional similarity, at least at the spatial scale that we studied. That said, it is possible that ECM composition and physiology are affected by soil processes at smaller spatial scales, as are other microbes (Schimel and Bennett 2004). The possibility that niche partitioning occurs at finer spatial scales than at the site level was suggested by differences we observed in ECM richness and taxon abundance between soil layers, supporting Dickie et al. (2002). We selected only mesic, zonal sites, and including a broader range of site qualities (e.g., a comparison of different biogeoclimatic zones) across both small and large spatial scales may have resulted in different patterns between ECM community diversity, structure, and soil nutrients. Other soil attributes not tested in this study, such as pH, micronutrients, moisture potential, and in situ nitrogen availability over time, may also play important roles in ECM community structure. Differences in inoculum availability, competition among ECM fungi, non-ECM plant community structure, as well as variation in vigor or genotypes among host plants (Whitham et al. 2006), were likely responsible for much of the variation in ECM diversity and community composition that were not accounted for by stand age or soil attributes.

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