Ecology, 83(1), 2002, pp. 104–115

BELOWGROUND ECTOMYCORRHIZAL FUNGAL COMMUNITY CHANGE OVER A NITROGEN DEPOSITION GRADIENT IN ALASKA

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Abstract. Nitrogen availability may be a major factor structuring ectomycorrhizal fungal communities. Atmospheric nitrogen (N) deposition has been implicated in the decline of ectomycorrhizal fungal (EMF) sporocarp diversity. We previously characterized the pattern of decreased sporocarp species richness over an anthropogenic N deposition gradient in Alaska (USA). To determine whether this change in sporocarp community structure was paralleled below ground, we used molecular and morphological techniques to characterize the ectomycorrhizal community of white spruce (*Picea glauca*) over this gradient. We then related patterns of richness and relative abundance of taxa to various N-affected environmental parameters. Species richness of EMF declined dramatically with increasing N inputs. Over 30 taxa were identified at the low-N sites, compared with nine at the high-N sites. Low-N site dominants (*Piloderma* spp., *Amphinema byssoides*, *Cortinarius* spp., and various dark-mantled *Tomentella* spp.) disappeared completely at the high-N sites, where they were replaced by *Lactarius theiogalus*, *Paxillus involutus*, *Tylospora fibrillosa*, *Tomentella sublilacina*, *Thelephora terrestris*, and an unidentified species. *Lactarius theiogalus* accounted for 44–68% of the root tips at the high-N sites, compared with 7–20% of tips at the low-N sites. Organic horizon mineral N and foliar nutrient ratios (N:P, P:Al) were excellent predictors of taxonomic richness ($r^2 > 0.93$). Organic horizon NO_3^- availability was the best predictor of abundance of many taxa. These patterns suggest that long-term N deposition can lead to decline in EMF species richness, and dramatic changes in EMF community structure. The consequences of these changes for plant nutrition and ecosystem function depend on how EMF community function changes as community structure changes. We speculate that as N inputs increase, the EMF community shifts from taxa specialized for N uptake under low-N conditions (e.g., *Cortinarius*, *Piloderma*), toward taxa specialized for high overall nutrient availability (e.g., *Tomentella sublilacina*, *Thelephora terrestris*) and finally toward taxa specialized for P uptake under high-N, low-P, acidified conditions (e.g., *Paxillus involutus*, *Lactarius theiogalus*).

Key words: acidification; Alaska; ectomycorrhizal fungi; foliar nutrients; mineralization; nitrification; nitrogen deposition; PCR; RFLP; sequence analysis; soil nitrogen; species richness.

INTRODUCTION

Ectomycorrhizal fungi (EMF) play a critical role in tree nutrition and carbon balance, supplying soil resources to their plant hosts in exchange for sugars (Smith and Read 1997). Ectomycorrhizal trees dominate nitrogen-limited forest ecosystems, and EMF vary in their nitrogen uptake physiology (Smith and Read 1997, Chalot and Brun 1998), so we might expect different species to dominate in soils with different levels and forms of nitrogen.

Sporocarp analysis suggests that EMF species richness declines in response to nitrogen fertilization and atmospheric N deposition (Wallenda and Kottke 1998 and references therein; Lilleskov et al. 2001). However, sporocarp communities are not directly representative of belowground communities (Mehmann et al. 1995, Gardes and Bruns 1996*a*). EMF species may shift their C allocation away from fruiting in response to N fertilization (Godbout and Fortin 1990), while remaining otherwise unaffected below ground. Furthermore, many important EMF species do not form conspicuous aboveground sporocarps (e.g., Gardes and Bruns 1996*a*). The effect of nitrogen additions on belowground EMF community structure has received little study, largely because of the difficulty of characterizing the EMF community below ground. Earlier studies investigating N fertilization responses utilized morphological identification techniques (morphotyping), which do not permit identification of many of the fungal symbionts, and which often lead to false lumping and splitting of taxa (Mehmann et al. 1995, Kårén and Nylund 1997).

Manuscript received 1 May 2000; revised 26 October 2000; accepted 6 November 2000; final version received 20 February 2001.

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Recently, molecular methods have been used to identify EMF in N-fertilized conifer plantations in Sweden (Kårén 1997, Kårén and Nylund 1997). However, no studies have used molecular approaches to study the EMF community response in natural forests, and none has examined the long-term response to increased atmospheric N deposition (as distinct from pulsed fertilization). The effects of fertilization and atmospheric deposition may differ because of the pulsed nature of fertilization inputs, in contrast with the chronic low-level inputs from atmospheric deposition (Johnson 1992).

To determine the effect of N deposition on EMF communities below ground, we used molecular methods to examine the EMF community composition at a subset of sites from a nitrogen deposition gradient over which the pattern of EMF fruiting had been characterized (Lilleskov et al. 2001). We hypothesized that there would be a decline in EMF belowground species richness at high-N sites, and that the community structure would shift in parallel with aboveground changes. We also examined the relationship of EMF species richness and community structure with specific environmental factors affected by N deposition.

Methods

The study area and general methods for sampling and analysis of sporocarps, soil chemistry, foliar chemistry, tree growth, and plant community composition have been described in detail elsewhere (Lilleskov et al. 2001). Briefly, the nitrogen source was gaseous emissions of ammonia from a fertilizer facility near Nikiski, on the Kenai Peninsula of Alaska, USA (Fig. 1). The facility had been in operation since 1968. In 1992, bulk deposition was estimated at ~20 kg $N \cdot ha^{-1} \cdot yr^{-1}$ at the high-N end of the gradient, and ~1 kg $N \cdot ha^{-1} \cdot yr^{-1}$ at the low-N end of the gradient (Whytemare et al. 1997). Historic N inputs were considerably higher than present inputs, as control measures were implemented in the mid-1980s, reducing emissions by ~80% (Sullivan et al. 1990; D. Newbold, *personal communication*).

Sampling for this part of the study was carried out in July–September 1995, as part of a study that also examined patterns of fungal fruiting across the gradient (Lilleskov et al. 2001). A subset of five sites was used in this study, from 15 used in the sporocarp study (Fig. 1). Two of these sites were subject to heavy N deposition, one was intermediate, and two others received low deposition. All were all located on well-drained, fine sandy loam soils. Sites were all dominated by mature white spruce (*Picea glauca* (Moench) Voss) stands between 110 and 150 yr old, with Alaska paper birch (*Betula kenaica* Evans) subdominant and small amounts of *Populus* spp. also present. We selected three focal spruce trees (20–35 cm dbh) per site for root sampling, from the five trees per site used in the previous study (Lilleskov et al. 2001).

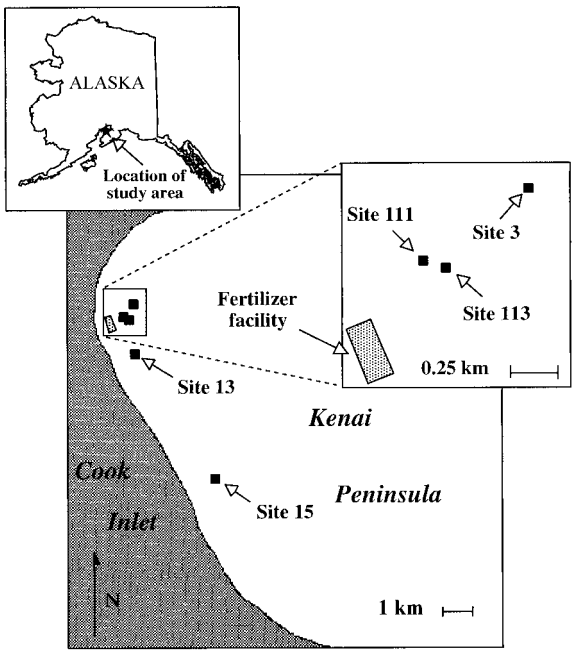

FIG. 1. Map of the study area north of Kenai, Alaska. Nitrogen deposition is heaviest to the NNE of the fertilizer facility. Sites are labeled with site numbers. The area of detail shows the downwind sites.

Ectomycorrhizal root sampling.—Spruce roots were sampled in mid-September 1995. At each focal tree, a main root at the base of the trunk was selected randomly and was followed along one face until a branch root was found. This was traced until short roots were encountered and were gently separated from the bulk soil. Tracing was continued to the end of the root. This process was repeated three times for each main root, on three main roots per tree, for a total of nine separate roots traced per tree. Only roots from the organic horizon and surface of the mineral soil were traced, because roots deeper than the surface mineral horizons were too difficult to excise intact. The samples were pooled for each tree and frozen at −20°C.

Identification of ectomycorrhizas.—To identify ectomycorrhizas, we used a combination of morphological and molecular genetic methods. Frozen roots were transferred to ethanol prior to sorting in order to preserve fungal DNA. Roots were placed in 50% ethanol for 1 h, transferred to 75% ethanol for 1 h, and finally transferred to 100% ethanol. These roots were gently cleaned of surrounding soil and were cut into short (~1–2 cm) lengths prior to subsampling. From each tree, 300 tips were randomly subsampled and sorted into morphotypes using criteria of Agerer (1987–1996): hyphal anatomy and color, rhizomorph characteristics, mantle color and surface characteristics, and ectomycorrhizal morphology and branching patterns (Appendix A). When possible, EMF were identified by this method. For some species, identification was possible

for some tips but not others, e.g., *Piloderma* spp. (which in some phases form distinctly yellow hyphae, but in other phases are white); and *Amphinema byssoides* (which sometimes forms distinctive rhizomorphs). For these types, we used morphology to identify the fungus when distinctive characters were present, and then used molecular methods to confirm the identity of tips lacking the distinctive characters, and to check our identifications.

For molecular identification, all morphotypes from each tree were subsampled, with each subsample consisting of one to three root tips. The number of subsamples varied from one to three depending on the abundance of the morphotype. When more than one subsample was taken per morphotype, subsamples were selected to maximize the morphological variation among subsamples. This variation was regarded as insufficient to justify separation into distinct morphotypes, e.g., slight shifts in color, mantle shininess, profuseness of hyphae, etc. The rationale for this sampling regime was to maximize the likelihood of encountering variation within morphotypes during the initial subsampling round, in case morphotypes represented mixtures of taxa.

To identify the fungi using molecular methods, two approaches were used. First, root tips were analyzed for patterns of restriction fragment length polymorphism (RFLP) in the internal transcribed spacer (ITS) region of nuclear DNA coding for ribosomal RNA (rDNA) (Gardes and Bruns 1996*b*), and these RFLP patterns were compared to those of sporocarps. Briefly, DNA was extracted from root tips and sporocarps, purified, and the ITS was amplified by polymerase chain reaction (PCR), using the primers ITS1-F and ITS4 (White et al. 1990, Gardes and Bruns 1993). These primers selectively amplify fungal DNA without amplification of the plant DNA. This DNA was then cut using the restriction enzymes *Dpn*II, *Hin*fI, and (when necessary for identification) *Alu*I. The digested fragments were separated by gel electrophoresis. DNA fragment lengths were determined using the NCSA software Gel Reader 2.05 (National Center for Supercomputing Applications, Champaign, Illinois, USA). Putative matches were electrophoresed alongside DNA from identified fungi for final determination of identity.

Second, if restriction patterns did not match sporocarps or EM identified morphologically, fungi were identified using sequence analysis of either a portion of the mitochondrial large subunit (Bruns et al. 1998) or the ITS. Mitochondrial DNA was amplified using the primers ML5 and ML6. For the mitochondrial DNA, sequences were compared with a large database developed by Bruns et al. (1998), using the phylogenetic analysis program PAUP 4.0b4a for Macintosh (Swofford 2000). For this purpose, we used a bootstrap analysis of a neighbor joining tree, with 1000 replicates and groups with >70% support retained. This approach allows identification of taxa to varying degrees of taxonomic resolution; i.e., some regions of the trees generated are quite well resolved taxonomically (to genus, family, or order), whereas others are not. Sequences that fell into these distinct, well-supported taxonomic groups were given a separate number for each type, e.g., Thelephoroid 1 for species in the Thelephorales. Greater resolution (to genus or species) was possible for some taxa by comparing ITS sequences with known ITS sequences, also in PAUP. ITS sequences were available for *Tylospora* (Eberhardt et al. 1999), Thelephoraceae (Kõljalg et al. 2000), and *Piloderma* (E. Larsson, *unpublished data*). Accession numbers for all sequences are listed in Appendix B. When no ID was possible for a distinct RFLP type via morphological methods, ITS-RFLP matching, or sequencing, it was identified as RFLP followed by a number (e.g., RFLP 17).

Statistical analysis.—The relationship between EMF diversity and the environmental variables was examined at the site level. Comparisons involved linear correlations and linear and quadratic regressions of environmental variables on taxonomic richness for the total community, and richness and relative abundance of specific taxonomic groups. Relative abundance for a taxon is the percentage that it contributes to the total number of tips sampled at a site. The number of taxa and the percentage abundance were log-transformed to improve the homoscedasticity of the data and to linearize relationships with predictor variables. All statistical analyses were carried out on Minitab 10.5 (Minitab 1995) on a Macintosh computer.

Results

EMF identification

We found 49 distinct RFLP types across the five sites (Appendix C). Most types were identified to some level of taxonomic resolution. Twelve RFLP types (comprising 45% of the root tips) were identified to the species or subgenus level using RFLP matching to sporocarps; 17 RFLP types (comprising 37% of the root tips) were identified to species, family, or order using sequence information; two RFLP types (comprising 6% of the root tips) were identified to genus using morphology, and their uniformity was confirmed with RFLP; and one morphotype (comprising 3% of the root tips) was identified by morphology alone. Eleven RFLP types (comprising 9% of the root tips) did not match sporocarp RFLP patterns and were not sequenced. Although they could not be identified, the abundance of these RFLP types could be compared across sites. Approximately 1% of the tips were in morphotypes for which no amplification was successful.

In most cases in which matches to sporocarps were successful, EMF on root tips could be identified to species, with the exception that many species in *Cortinarius* subgenus *Telamonia* were indistinguishable based on their RFLP patterns, and so were pooled into

TABLE 1. Environmental variables (mean ± 1 SE) measured at five sites over an atmospheric N deposition gradient near Kenai, Alaska.

	High N ←				→ Low N
Environmental variable	Site 111	Site 113	Site 3	Site 15	Site 13
Bulk N deposition ($kg \cdot ha^{-1} \cdot 60\ d^{-1}$)	2.3	1.8	1.0	0.15	0.38
Organic horizon N					
Net nitrification ($mg\ N \cdot kg\ soil^{-1} \cdot 28\ d^{-1}$)	167 ± 37	119 ± 27	109 ± 10	1.4 ± 0.6	0.4 ± 0.2
Net mineralization ($mg\ N \cdot kg\ soil^{-1} \cdot 28\ d^{-1}$)	210 ± 40	173 ± 55	244 ± 20	92.3 ± 87.8	42.0 ± 20.2
KCl-extractable NO_3^--N (mg/kg soil)	67.9 ± 16.4	41.0 ± 20.8	31.5 ± 5.2	0.03 ± 0.1	0.00 ± 0.0
KCl-extractable mineral N (mg/kg soil)	243 ± 25	168 ± 38	92.8 ± 14.6	13.3 ± 3.7	13.3 ± 6.1
Anion exchange NO_3^--N ($mg \cdot bag^{-1} \cdot 28\ d^{-1}$)	3.52 ± 0.58	3.63 ± 1.28	2.63 ± 1.87	0.08 ± 0.01	0.06 ± 0.01
Soil pH					
Minimum soil pH ($CaCl_2$)	3.84 ± 0.04	3.88 ± 0.05	4.01 ± 0.03	4.33 ± 0.02	4.23 ± 0.02
Organic soil pH ($CaCl_2$)	3.40 ± 0.01	3.44 ± 0.06	3.41 ± 0.03	3.84 ± 0.06	3.77 ± 0.07
Foliar chemistry					
N (g/kg)	16.7 ± 1.7	16.0 ± 0.3	15.2 ± 0.9	13.2 ± 0.3	13.9 ± 0.7
K (g/kg)	6.99 ± 0.93	7.73 ± 0.28	7.21 ± 0.50	9.14 ± 0.35	8.38 ± 0.97
P (g/kg)	1.62 ± 0.21	1.50 ± 0.01	1.91 ± 0.11	1.95 ± 0.05	2.39 ± 0.16
Ca (g/kg)	1.93 ± 0.02	1.56 ± 0.14	2.23 ± 0.17	2.61 ± 0.55	2.44 ± 0.20
Mg (g/kg)	0.77 ± 0.11	0.83 ± 0.04	0.90 ± 0.11	0.93 ± 0.05	0.86 ± 0.04
Al (mg/kg)	30.1 ± 5.1	24.0 ± 0.9	21.1 ± 1.0	19.5 ± 0.6	20.5 ± 0.4
N:P	10.4 ± 0.95	10.7 ± 0.18	7.96 ± 0.05	6.76 ± 0.26	5.84 ± 0.24
N:Mg	22.6 ± 4.0	19.4 ± 0.9	17.6 ± 2.8	14.2 ± 0.6	16.1 ± 0.6
Mg:Al	25.5 ± 9.4	34.5 ± 0.6	42.6 ± 7.1	47.6 ± 4.4	42.1 ± 1.0
P:Al	53.7 ± 20.0	62.5 ± 1.8	90.4 ± 4.9	100.0 ± 4.7	116.6 ± 8.6
Plant community					
Feather moss cover (%)	3.3 ± 1.7	3.3 ± 0.8	2.5 ± 1.4	70.0 ± 5.8	66.7 ± 14.5
Grass relative abundance (%)	63.3 ± 18.4	51.7 ± 3.4	46.0 ± 13.1	25.3 ± 1.3	8.7 ± 1.8
Spruce basal area (m^2/ha)	15.0 ± 7.6	26.7 ± 2.9	16.7 ± 3.6	27.5 ± 2.9	24.2 ± 2.7
Birch basal area (m^2/ha)	0.8 ± 0.8	2.5 ± 1.4	7.5 ± 1.4	0.0 ± 0.0	10.4 ± 2.9

several different types within the subgenus. Because this subgenus contained a large portion of the diversity in the sporocarp community at the low-N sites, species richness at these sites has probably been underestimated in the following analysis.

Sequence analysis.—For RFLP types unmatched to sporocarps, phylogenetic analysis of the mitochondrial sequences placed most unknowns in the Thelephorales or Russulaceae (91% and 98% support in the bootstrap analysis, respectively). These taxonomic groups were very well supported in tests of the initial data set (Bruns et al. 1998), and were supported by the bootstrap analysis. As a result, there is high confidence in the identity of these taxa. Subsequent RFLP matches to sporocarps confirmed the ML5-ML6 sequence placement of *Russula burkei* in the Russulaceae and *Laccaria bicolor* in *Laccaria* sp.; subsequent ITS sequence analysis placed many of the Thelephorales in the genus *Tomentella* or *Thelephora* (Thelephoraceae; U. Kõljalg, *personal communication*).

For one group of unmatched RFLP types, ITS sequence matches confirmed their identity as *Tylospora fibrillosa*. ITS sequence similarity was the same between the unknowns and European *T. fibrillosa* (97.5–99.5%) as it was among the European *T. fibrillosa* isolates (97.5–100%).

Several unmatched RFLP types believed to be *Piloderma* spp., based on morphological evidence, were confirmed as *Piloderma fallax* (Libert) Stalpers (=*P. croceum* Eriksss. et Hjörts. and *P. bicolor* (Peck) Jülich) and *P. byssinum* (Karst.) Jülich, based on sequence similarity to European collections (E. Larsson, *personal communication*).

Pooling of unmatched RFLP types.—In three cases, multiple RFLP types were considered to represent intraspecific variation, and so were pooled for later analyses. In the first case, based on similarity of the RFLP patterns, ITS sequences, and morphology, five RFLP types were believed to be intraspecific variants of *Tylospora fibrillosa*. In the second case, based on morphological data and ITS sequence similarity, three RFLP types were believed to be *Piloderma fallax*. The mycorrhizas of the most abundant type exhibited both bright yellow and white tips, with white dominant, and had ≥98% ITS sequence similarity with the two rarer types, which were morphologically similar but all white. Third, Thelephoraceae 1 and 1a were considered to be the same type because they were morphologically indistinguishable and their RFLP patterns differed only in the addition of one faint band.

Environmental factors

Environmental factors varied across the gradient in predictable ways as atmospheric N inputs increased (Table 1). In particular, all measures of inorganic N availability in the organic horizon were much higher at the high-deposition sites than at the low-deposition sites. As soil N increased, soil pH declined nearly half a unit, and foliar N, N:P, N:Mg, and Al increased, whereas foliar P, base cations, P:Al, and Mg:Al de-

TABLE 2. Relative abundance (%) of ectomycorrhizal fungal taxa on root tips, over an atmospheric N deposition gradient near Kenai, Alaska.

	High N ←				→ Low N
Taxon	Site 111	Site 113	Site 3	Site 15	Site 13
Paxillus involutus (Batsch:Fr.) Fr.	18.4	5.2	3.3		
Lactarius theiogalus (Bull.:Fr.) S.F. Gray	68.5	43.9	25.7	6.3	7.4
Basidiomycete 1	7.8	6.1	4.3	3.2	1.2
Tomentella sublilacina (Ellis & Holw.) Wakef.	0.7	25.2	33.7	6.1	3.6
Tylospora fibrillosa (Burt) Donk	1.3	4.2	15.7	7.2	1.9
Cenococcum geophilum Fr.	2.0	0.8	1.0	7.4	4.8
RFLP 37		0.1			
Thelephora terrestris Fr.		12.1	5.4		
Hebeloma mesophaeum (Pers.) Quél.		2.1	3.3		
Laccaria bicolor (Maire) Orton			1.2		
RFLP 13			1.0		
Lactarius olivaceo-umbrinus Smith			0.7		
Russuloid 1			0.3		
RFLP 36			0.3		
Cortinarius subg. *Telamonia* 1			1.7	15.0	0.8
Amphinema byssoides (Pers.:Fr.) Erikss.			1.9	5.8	14.8
Piloderma fallax (Libert) Stalpers			0.5	9.8	1.7
Tomentella subclavigera Litsch.				1.2	
Tomentella sp. 1				1.2	
Cortinarius subg. *Telamonia* 3				1.1	
Russuloid 3				1.1	
RFLP 16				7.6	0.3
Tomentella stuposa (Link) Stalpers				6.5	0.2
Genea sp.				6.1	0.8
Cortinarius subg. *Telamonia* 2				6.0	3.1
Thelephoroid 3				3.6	7.5
Tricholoma inamoenum (Fr.:Fr.) Gill.				2.6	6.5
Piloderma byssinum (Karst.) Jülich				1.6	24.1
Basidiomycete 2					7.9
Thelephoroid 7					3.7
Thelephoroid 4					2.5
Cortinarius obtusus gp.					1.6
Cortinarius subg. *Telamonia* 4					1.1
Russula burkei Burlingham					1.0
RFLP 34					0.8
Tomentella sp. 2					0.3
Thelephoroid 9					0.3
RFLP 17					0.3
RFLP 20					0.1
RFLP 33					0.1
RFLP 35					1.1
Unidentified	1.2	0.2		0.7	1.1

Notes: Sites are ordered from high to low soil N status. Taxa are ordered from high-N site dominants to low-N site dominants.

creased. As N inputs increased, grass increased from minimal cover to dominance of the herb layer, and feather mosses declined correspondingly. Not surprisingly, many of these environmental factors were highly correlated.

EMF taxonomic richness and site N status

There was a strong decline in taxonomic richness as the soil N availability increased. The two low-N sites had three times more taxa than did the high-N sites (Table 2). Many of the N-affected environmental factors were excellent predictors of overall taxonomic richness and richness in species-rich taxonomic groups (Thelephorales and Cortinariaceae). For example, richness declined with increasing N availability (foliar N:P, organic horizon extractable mineral N), acidification (foliar P:Al), and grass abundance (Table 3).

Relative abundance of EMF taxa and site N status

The best overall predictor of the relative abundance of individual taxa was organic horizon net nitrification (Fig. 2). Other measures of soil nitrate availability (extractable NO_3^-, anion exchange NO_3^-), plant N status (foliar N, N:P), acidification (soil pH, foliar P:Al), and herbaceous vegetation (moss cover, graminoid abundance) were also excellent predictors of the abundance of many taxa (Appendix D, *Ecological Archives*).

As site N status increased, there were three general patterns of relative abundance for individual taxa: declining, peaked (i.e., highest at intermediate N inputs),

TABLE 3. Linear correlations between environmental variables and number of ectomycorrhizal fungal taxa (log-transformed) over a nitrogen deposition gradient near Kenai, Alaska.

Environmental variable	Total	ECMF taxonomic group	
		Thelephorales	Cortinariaceae
Precipitation N inputs			
Bulk N deposition	−0.93	−0.91	−0.96
Organic soil N			
KCl-extractable mineral N	−0.96	−0.93	−0.98
KCl-extractable NO_3^--N	−0.93	−0.96	−0.97
Net nitrification	−0.89	−0.98	−0.92
Foliar nutrients			
P:Al	0.99	0.91	0.93
N:P	−0.97	−0.90	−0.91
Plant community			
Grass relative abundance	−0.95	−0.99	−0.92
Feather moss cover	0.78†	0.93	0.77†

Notes: Results are presented for total number of taxa and for the two most diverse taxonomic groups. For all correlations, $n = 5$. All correlations are significant at $P < 0.05$, unless otherwise indicated.

† Correlations are not significant; $P > 0.05$.

and increasing. For ease of communication, we use the terms nitrophobic and nitrophilic to describe the declining and increasing groups, respectively, even though the effect may be only indirectly attributed to N. Most taxa fell into the nitrophobic group (Table 2, Fig. 2). Of the taxa common at the low-N sites, *Piloderma* spp., *Amphinema byssoides*, all *Cortinarius* species, *Cenococcum geophilum*, and all dark, rough-mantled Thelephoroid (probably all *Tomentella*) species declined or disappeared with increasing N status (Table 2, Fig. 2f–i). In contrast, *Lactarius theiogalus*, *Paxillus involutus*, and Basidiomycete 1 all increased with increasing N status (Table 3, Fig. 2a–c). One taxon peaked at moderate N levels (*Tomentella sublilacina*; Fig. 2e), and another showed no significant patterns (*Tylospora fibrillosa*; Fig. 2d).

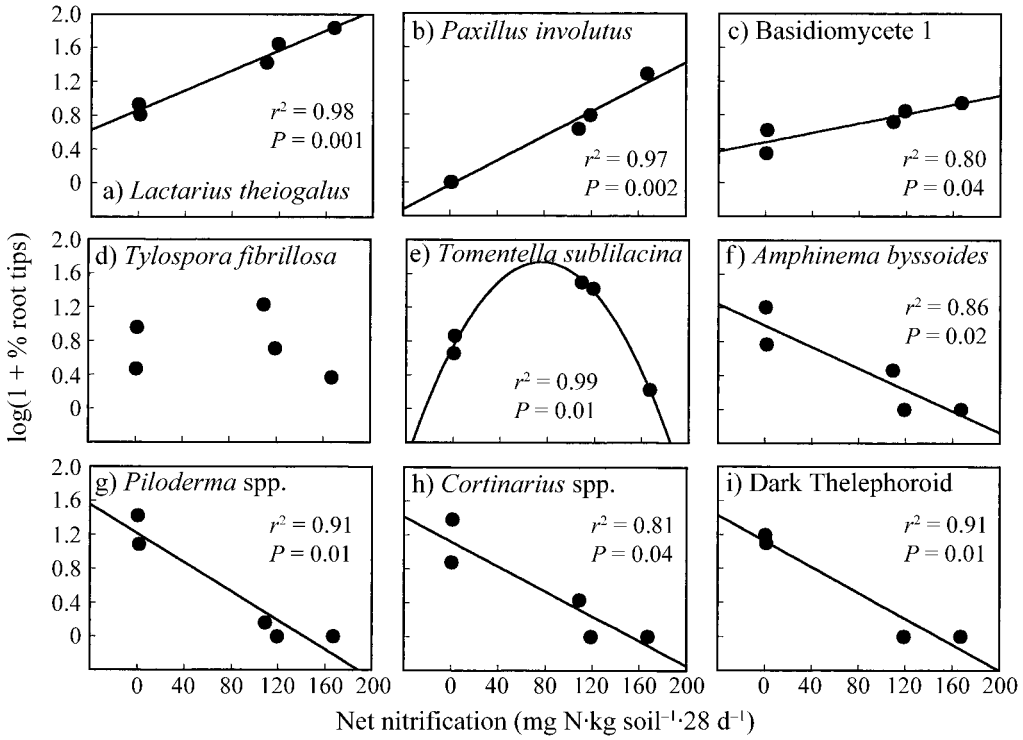

FIG. 2. Regressions of percentage of root tips (log-transformed) vs. net nitrification for individual ectomycorrhizal fungal taxa over an atmospheric nitrogen deposition gradient near Kenai, Alaska. For all regressions, $n = 5$.

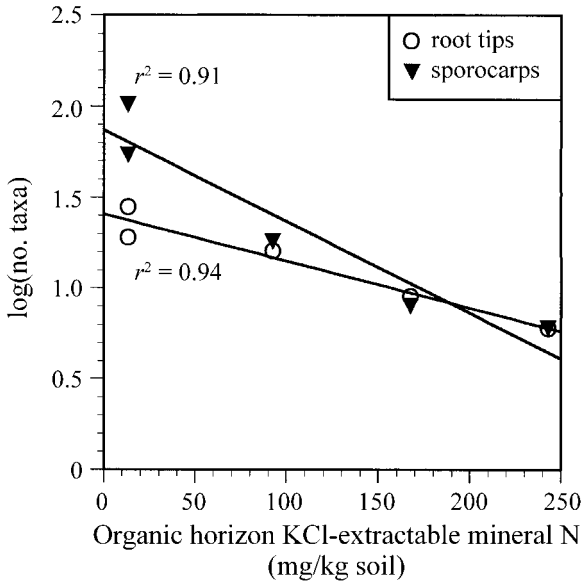

FIG. 3. Regression of sporocarp (triangles) and root tip (circles) taxonomic richness (log-transformed) vs. organic horizon mineral N for ectomycorrhizal fungi over an atmospheric nitrogen deposition gradient near Kenai, Alaska.

Sporocarps vs. belowground EMF taxonomic richness

There were certain parallels between the above- and belowground measures of species richness and abundance. Both sporocarp and belowground measures of species richness declined with increasing soil N (Fig. 3). The slopes of the relationships differed, largely because one low-N site had much lower belowground species richness than sporocarp species richness (Fig. 3). This occurred primarily because of the high number (59) of *Cortinarius* species fruiting at that site, compared with the low number (4) of *Cortinarius* RFLP types. Most species in the dominant subgenus (*Telamonia*) were unresolved using our methods, so the actual belowground richness of *Cortinarius* at these sites was probably much higher.

DISCUSSION

The results of the present study are consistent with the hypothesis that long-term N deposition can cause declines in belowground EMF species richness and dramatic changes in EMF community composition and structure. Although we must use caution in interpreting causation from a correlative study, given the strong correlations of the community change with N-affected variables, the most parsimonious explanation is that these changes are a response to some factor or combination of factors affected by N inputs.

This interpretation is supported by the only other molecular genetic study on the response of EMF communities to long-term N fertilization. In a study carried out in a Scotch pine (*Pinus sylvestris*) plantation in northern Sweden fertilized for 24 yr, the EMF community change in response to N fertilization was strikingly similar to the present study (Kårén 1997). Fertilization reduced the number of RFLP types by 50% relative to controls. The percentage of roots colonized by *Tylospora fibrillosa* and *Lactarius rufus* increased, and the percentage colonized by various *Cortinarius* species and *Piloderma croceum* decreased.

In contrast, three short-term molecular studies of fertilization effects found large community responses when sporocarps were examined, but relatively little community response below ground (Kårén and Nylund 1997, Jonsson et al. 2000, Peter et al. 2001). Kårén and Nylund (1997) did note a significant increase in a smooth brown morphotype, which was largely *T. fibrillosa*, but also included *Lactarius theiogalus* and several other taxa. This is consistent with our finding of an increase in smooth, pale brown-mantled taxa (*T. fibrillosa, L. theiogalus, and Tomentella sublilacina*) at the intermediate-to-high-N levels. Peter et al. (2001) noted that the response in the sporocarp community was greater than in the belowground community, but did detect a decline in the belowground frequency of *Russula* species after only two years of fertilization, whereas a dominant Thelephoraceae species and *Tylospora asterophora* were unaffected.

Other studies of fertilization effects on EMF communities used morphotyping only, which makes comparison with the present study difficult. Two studies found a significant decrease in *Cenococcum geophilum* in response to fertilization (Menge and Grand 1978, Gill and Lavender 1983). Both Saunders et al. (1996) and Brandrud and Timmermann (1998) found no evidence of a change in morphotype composition after five years of fertilization.

In summary, results of the present study and those of Kårén (1997) seem compelling in regard to the effects of long-term N inputs on natural EMF communities. This suggests that long-term fertilization and atmospheric N deposition may have similar effects on EMF community structure, and that the pattern of response may be similar in oligotrophic conifer forests across the boreal forest zone. Short-term studies suggest that, compared to the relatively rapid decline in sporocarp abundance and diveristy in response to fertilization, the belowground community response is slower.

Comparison of response in sporocarp and belowground EMF communities

Across all 15 sites in the sporocarp study, there was a clear pattern of declining sporocarp diversity strongly correlated with measures of soil N and base status (Lilleskov et al. 2001). Epigeous sporocarps are easier to sample and identify than mycorrhizas; therefore, it is worth knowing whether they are good indicators of belowground community composition and structure. The primary reason why conspicuous epigeous spo-

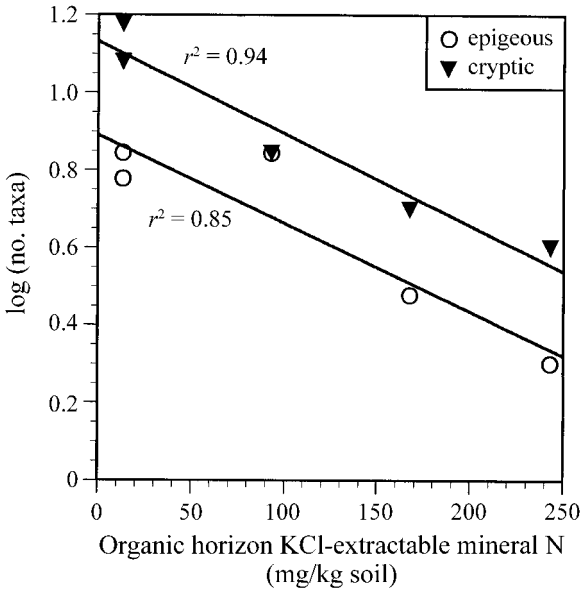

FIG. 4. Regression of taxonomic richness on root tips (log-transformed) vs. organic horizon mineral N for taxa producing either conspicuous epigeous sporocarps (circles) or cryptic sporocarps (triangles) over an atmospheric nitrogen deposition gradient near Kenai, Alaska.

rocarps might not be perfect indicators of belowground species richness is that taxa producing inconspicuous sporocarps (*Piloderma* spp., *Tylospora fibrillosa*, *Amphinema byssoides*, and Thelephoraceae), are an important component of the belowground community, especially at the low- and medium-N sites (Table 2). However, belowground declines occurred both in taxa with conspicuous epigeous sporocarps and in the more cryptic taxa (Fig. 4), suggesting that the aboveground fruiting patterns may be good indicators of overall trends in belowground species richness, at least at sites with long-term N deposition.

A second reason that sporocarps might be imperfect indicators is that the N effect on allocation to fruiting may vary among EMF taxa. For the nitrophobic taxa, the relationship between sporocarp abundance and belowground EMF abundance appeared to be stronger than for nitrophilic taxa. Patterns of belowground abundance of some nitrophobic taxa closely paralleled their aboveground abundance (e.g., *Tricholoma inamoenum*, $r^2 = 0.999$, $P < 0.001$; and *Cortinarius*, $r^2 = 0.93$, $P = 0.009$). In the case of *Cortinarius*, comparison was at the genus level because of lack of clear resolution among species in *Cortinarius* subgenus *Telamonia*. In contrast, for the dominant nitrophilic species *Lactarius theiogalus*, *Laccaria* spp., and *Paxillus involutus*, there was a weaker relationship between sporocarp and belowground abundance as N increased (Table 4). This may reflect more plasticity in allocation to fruit body production for the nitrophilic taxa. The weak relationship could arise from decreased host carbon (C) allocation to roots (Björkman 1949, Ericsson 1995), increased C costs of assimilation of NH_4^+ into C skeletons (Wallander 1995), or lower fungal allocation to reproductive vs. vegetative structures. Reduced allocation to fruiting under high-N conditions, despite an increase in the percentage of root tips colonized, could reflect a shift toward vegetative colonization of root tips under conditions favorable for nitrophilic taxa.

Our interpretation of the relationship of sporocarp production and ectomycorrhizal root tips may have been confounded by the presence of an alternate host (birch). However, in a pine plantation, Kårén (1997) similarly found that fertilization resulted in a decrease in the fruiting of *Lactarius rufus*, despite an increase in the percentage of root tips colonized by that species.

Laccaria bicolor sporocarps were much more abundant than their EM, consistent with the observations that *Laccaria* species often fruit prolifically from relatively few EM (Godbout and Fortin 1990, Ingleby et al. 1990), suggesting a high proportional allocation to reproduction.

Effect of N fertilization and acidification

In the present study, the driving force for EMF community change was atmospheric N deposition. The causal relationship between high N inputs to forest ecosystems and other environmental factors is relatively well understood (Aber 1992, Aber et al. 1998), and the expected responses (increased N mineralization and nitrification, acidification, altered plant nutrition, and changes in herbaceous plant community composition) were seen in the present study. Less clear is which of the specific N-affected variables led to the change in EMF community structure and composition. We will examine the evidence for N fertilization and acidification effects on EMF, and then consider some of their possible interactions.

Many EMF taxa appear to be sensitive to nitrogen rather than acidification. Sporocarp production by *Cortinarius*, *Russula*, and *Tricholoma* has declined in response to relatively short-term N fertilization (Shubin et al. 1977, Ohenoja 1978, 1989, Termorshuizen 1993, Brandrud 1995), before declines in soil pH were likely,

TABLE 4. The relationship between aboveground (sporocarp) and belowground (root tip) relative abundance for ectomycorrhizal fungal taxa over a nitrogen deposition gradient near Kenai, Alaska.

Taxon	r^2	P
Nitrophobic taxa		
Cortinarius spp.	0.93	<0.01
Russula spp.	0.96	<0.01
Tricholoma inamoenum	0.999	<0.001
Nitrophilic taxa		
Hebeloma mesophaeum	0.40	NS
Laccaria spp.	0.37	NS
Lactarius theiogalus	0.44	NS
Paxillus involutus	0.33	NS

Notes: For all regressions, $n = 5$. NS, not significant; $P > 0.05$.

although their belowground response to short-term fertilization is unknown. *Cortinarius, Piloderma, Russula,* and *Tricholoma* are typically associated with later stages of stand development (Visser 1995), in which N mineralization rates are lower and soil pH has declined (Van Cleve and Viereck 1981), so it seems likely that these taxa are adapted to relatively acidic, N-poor conditions.

Many of the nitrophilic fungi (e.g., *P. involutus, Thelephora terrestris, Laccaria* spp., *Hebeloma* spp.) colonize roots and fruit rapidly after trees are outplanted into former agricultural soils (e.g., Deacon et al. 1983, Mason et al. 1983, Dighton et al. 1986, Last et al. 1992), or after natural disturbance such as fire (Visser 1995). Under these conditions, inorganic nutrient availability and pH are relatively high (e.g., Van Cleve and Viereck 1981). Sporocarp production of several of these taxa (*Paxillus involutus, Lactarius theiogalus, Hebeloma* spp., and *Laccaria bicolor*) also has been found to increase in response to moderate, short-term N fertilization (Laiho 1970, Shubin et al. 1977, Ohenoja 1978, 1988, 1989, Salo 1979, Wasterlund 1982, Sagara 1992, Brandrud 1995), although the belowground response is not known.

Some taxa may be sensitive to acidification effects. *Amphinema byssoides* and dark-mantled *Tomentella* species (e.g., ''*Picearhiza nigra*'' of Agerer (1987–1996)) decline with increasing N inputs. Both respond positively to liming (Antibus and Linkens 1992, Taylor and Brand 1992, Veerkamp et al. 1997). Furthermore, *A. byssoides* is often found colonizing nursery seedlings (Danielson and Visser 1990, Ingleby et al. 1990, Grogan et al. 1994). This suggests that intolerance to soil acidification or nutrient imbalances, rather than nitrogen availability per se, may have led to its decline in the present study.

Similarly, positive effects of fertilization and negative effects of acidification (or nutrient imbalance) could give rise to the peak for some taxa at intermediate N levels. *Thelephora terrestris* and *Hebeloma mesophaeum* readily colonize seedlings in nurseries (Danielson and Visser 1990, Ingleby et al. 1990, Grogan et al. 1994). *Hebeloma* sporocarp abundance can be increased by addition of alkaline compounds (Sagara 1975, 1992, Kraepelin and Michaelis 1997). *T. terrestris* had high acid phosphatase activity, but grew best at high inorganic phosphorus (P_i) levels (Colpaert et al. 1997), suggesting that it may perform poorly as P_i availability decreases, e.g., in acid soils (Brady and Weil 1996).

At highest N inputs, *L. theiogalus* and *P. involutus* increase in importance. The combination of acidified soils and high N availability out of balance with other nutrients is rare in unpolluted forests, occurring only under high, long-term N inputs, e.g., in association with nitrogen fixing plants (Compton et al. 1997). A limited pool of EMF taxa is likely to be adapted to these conditions. Interestingly, *P. involutus* is frequently found with ectomycorrhizal N-fixing alders, *Alnus* spp. (Airaudi et al. 1993), and forms ectomycorrhizas with alders in vivo and in vitro (Murphy and Miller 1994, Ekblad et al. 1995). Although little is known about the natural conditions in which *L. theiogalus* thrives, it is associated with acidic soils (Hansen and Knudsen 1992) and does not grow with protein as a sole N source in pure culture (E. A. Lilleskov, *unpublished manuscript*).

Abundance of *P. involutus* was best predicted by organic horizon net nitrification. It has been shown to grow well with NO_3^- as an N source, in contrast with most EMF, which grow more effectively with NH_4^+ (Rapior et al. 1988, Finlay et al. 1992, Keller 1996). This ability could be important in N-enriched, acidified systems for two reasons. First, NO_3^- is highly available under these conditions and is a mobile anion, so less C would have to be expended in exploration for N, perhaps offsetting the cost of nitrate reduction. Second, utilization of NO_3^- leads to proton consumption in the mycorrhizosphere (Ulrich 1995), reducing rhizosphere acidification.

P. involutus may be specialized for high-N/low-P conditions. It seems to be more efficient at P_i uptake than N uptake (Ekblad et al. 1995, Högberg et al. 1999), and to have higher acid phosphatase activity (Pacheo et al. 1991) and P supply to seedlings (Wallander et al. 1997, Högberg et al. 1999) than other species.

Predicting EMF belowground community response to N deposition

In order to develop models to predict EMF community structure and composition, we must test the generality of the quantitative relationships between N-affected environmental factors and EMF community structure found in this study. We hypothesize that, for initially oligotrophic, N-limited forests, increases in soil NO_3^- availability will be highly correlated with changes in the EMF community structure, as measured by both sporocarp production (Lilleskov et al. 2001) and on roots. This should be true because excess nitrification is both an indicator of high N availability and a driver of ecosystem acidification; thus, it should be a good indicator of the suite of environmental changes likely to lead to change in EMF community structure.

Conclusions

The community patterns uncovered in this study strongly suggest that atmospheric N deposition can decrease belowground species richness of EMF. The complete reorganization of the EMF community structure in this system was very strongly related to a suite of N-affected environmental factors. Although we cannot specify which of these factors was causal, both increased soil N and acidification are likely to be important drivers of community change. We hypothesize a shift from dominance by oligotroph, nitrophobic taxa

specialized in efficient uptake of N under limiting conditions, to dominance by taxa specialized for conditions of high overall fertility, and finally a shift toward acid-tolerant nitrophiles with increasing adaptations for uptake of other nutrients, especially P.

The lack of perfect correspondence between sporocarp and belowground measures of EMF abundance means that sporocarps cannot be taken as strictly representative of community structure. However, sporocarps may be good indicators of potential community change; i.e., a decrease in the percentage of nitrophobic taxa fruiting may indicate that soils are becoming N enriched and that belowground community structure is likely to change. Further investigation of community dynamics over the course of fertilization is required to determine the lag time in belowground community response to fertilization when compared with sporocarp response.

Experimental manipulations are required to tease apart the relative importance of the different environmental factors in controlling EMF community dynamics. Our understanding of the suites of physiological and ecological traits of EMF must be deepened and integrated into models of EMF life history strategies. This will increase our understanding of both the basic ecology of these communities and the effect of changing EMF communities on ecosystem function.

Acknowledgments

We thank Ursula Eberhardt, Ola Kårén, Urmas Kõljalg, and Ellen Larsson for access to their sequences and help with identification, and Tom Bruns, Bill Ghiorse, and Ken Mudge for access to their labs. This research was supported by grants from NSF (Dissertation Improvement Grant DEB-9520760), the Andrew W. Mellon Foundation, the Kieckhefer Foundation, and Sigma Xi, and a graduate research assistantship from the Andrew W. Mellon Foundation.

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APPENDIX A

A table describing mycorrhizas formed by ectomycorrhizal fungi on white spruce over an atmospheric N deposition gradient in Alaska is available in ESA's Electronic Data Archive: *Ecological Archives* E083-002-A1.

APPENDIX B

A table providing Genbank accession numbers for sequences of ectomycorrhizal fungal PCR-amplified DNA from spruce ectomycorrhizas is available in ESA's Electronic Data Archive: *Ecological Archives* E083-002-A2.

APPENDIX C

A table providing ITS-RFLP band patterns for ectomycorrhizal fungi is available in ESA's Electronic Data Archive: *Ecological Archives* E083-002-A3.

APPENDIX D

A table showing linear correlations between environmental variables and the percentage of root tips occupied by ectomycorrhizal fungi is available in ESA's Electronic Data Archive: *Ecological Archives* E083-002-A4.