Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest

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Summary

• Our understanding of how saprotrophic and mycorrhizal fungi interact to recirculate carbon and nutrients from plant litter and soil organic matter is limited by poor understanding of their spatiotemporal dynamics.

• In order to investigate how different functional groups of fungi contribute to carbon and nitrogen cycling at different stages of decomposition, we studied changes in fungal community composition along vertical profiles through a *Pinus sylvestris* forest soil. We combined molecular identification methods with ^{14}C dating of the organic matter, analyses of carbon:nitrogen (C:N) ratios and ^{15}N natural abundance measurements.

• Saprotrophic fungi were primarily confined to relatively recently (< 4 yr) shed litter components on the surface of the forest floor, where organic carbon was mineralized while nitrogen was retained. Mycorrhizal fungi dominated in the underlying, more decomposed litter and humus, where they apparently mobilized N and made it available to their host plants.

• Our observations show that the degrading and nutrient-mobilizing components of the fungal community are spatially separated. This has important implications for biogeochemical studies of boreal forest ecosystems.

Key words: carbon, community, fungi, nutrient cycling, podzol, soil, terminal restriction fragment length polymorphism (T-RFLP).

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Introduction

Boreal forests cover *c.* 11% of the land surfaces of the earth and rival tropical rain forests as the largest terrestrial biome. They are characterized by cold climate, a long period of snow cover, slow decomposition of organic matter and poor plant nitrogen (N) supply (Persson, 1980). In boreal forests, fungi play pivotal roles in the circulation of carbon (C) and nutrients through the ecosystem. Saprotrophic fungi are the principal decomposers of wood and litter (Rayner & Boddy, 1988) and obtain energy by degrading dead organic matter. Mycorrhizal fungi, in contrast, obtain energy mainly as photoassimilates provided by symbiotically associated plants and in return provide their plant hosts with soil-derived nutrients (Smith & Read, 1997). More than half of the biological activity in boreal forest soils is driven by the supply of recently photosynthesized carbohydrates to roots, mycorrhizal fungi and other root-associated microorganisms (Högberg *et al.*, 2001). Although saprotrophic and mycorrhizal fungi are likely to play fundamentally different roles in the ecosystem, many ecological theories and methods treat them and other soil microorganisms as a single, ubiquitously occurring, functional group. Little is known about the spatiotemporal distribution of these functional groups of

fungi and how they interact during decomposition of plant litter.

We used DNA-based identification methods to study the vertical distribution and temporal succession of fungi within a boreal *Pinus sylvestris* forest soil. Typical boreal forest podzol soils are characterized by accumulation of organic matter on top of the mineral soil, and the absence of mixing soil animals leads to a profound vertical stratification, with the age of the organic matter increasing with depth (Trumbore & Harden, 1997). The temporal succession of fungi within the organic matter may therefore be studied by analysing the vertical distribution of fungal taxa within the soil. Previous studies of vertical niche differentiation among fungi have been limited to easily culturable saprotrophic microfungi (Söderström & Bååth, 1978) or ectomycorrhizal fungi (Dickie *et al.*, 2002; Landeweert *et al.*, 2003; Rosling *et al.*, 2003; Tedersoo *et al.*, 2003; Genney *et al.*, 2006). The present study encompasses the total fungal community, focusing on the upper organic soil horizons. Frequent switches between saprotrophism and symbiosis have occurred during evolution, and both strategies may be represented within groups of relatively closely related fungal taxa (Hibbet *et al.*, 2000). It is therefore necessary to use molecular techniques to analyse communities down to the level of genera and species in order to draw any conclusions about their functional potential. Here, polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) region of the ribosomal genes was combined with the community fingerprinting technique terminal restriction fragment length polymorphism (T-RFLP) (Dickie *et al.*, 2002). In order to identify taxa in the T-RFLP profiles, PCR products from representative samples were cloned, enabling sequencing of the ITS region of the ribosomal RNA (rRNA) genes for individual taxa within the mixed samples (Landeweert *et al.*, 2003; O'Brien *et al.,* 2005) and subsequent identification by database comparisons.

To investigate how different fungi influence C and nutrient cycling at different soil depths, we compared the fungal community composition in the samples with vertical patterns in soil C:N ratio and ^{15}N abundance (Nadelhoffer & Fry, 1988). Analysis of ^{14}C derived from nuclear bomb tests (Gaudinski *et al.*, 2001) enabled us to determine the age of the organic matter and convert the vertical gradient to a true temporal gradient.

Materials and Methods

Sampling and DNA extraction

Soil samples were collected from a *Pinus sylvestris* L. forest with an understory of ericaceous dwarf shrubs (*Vaccinium vitis-ideae* L. and *Calluna vulgaris* (L.) Hull) and mosses (*Pleurozium schreberi* (Bridel) Mitten and *Dicranum majus* Turner). The study was conducted in Jädraås IhV (60°49′ N, 16°30′ E, altitude 185 m), which is a well-documented (Persson, 1980) field site in central Sweden. In total, 27 soil cores (28 mm diameter) were collected and subdivided into eight vertical horizons each, according to Table 1. Soil samples were freeze-dried and ground in a ball mill. DNA was extracted from 50 to 100 mg of dry matter (500 mg of mineral soil fractions) in a CTAB buffer (3% cetyltrimetylammoniumbromid, 2 mM EDTA, 150 mM Tris-HCl and 2.6 M NaCl, pH 8) at 65°C for 1 h and precipitated from the supernatant with 2-propanol. The extracts were further purified through the Wizard Plus Minipreps DNA Purification System (Promega, Madison, WI, USA), the Geneclean Kit (Qbiogene, Irvine, CA, USA) or the QIAprep Spin Miniprep Kit (Qiagen, Venlo, the Netherlands), all of which are commercially available DNA cleaning kits.

PCR, cloning, sequencing and sequence analysis

DNA from all the different horizons was used for PCR, cloning and sequencing (Landeweert *et al.*, 2003; O'Brien *et al.,* 2005). In total, PCR products from 38 samples were

Table 1 Subdivision of soil cores into vertical horizons

Layer	Description	Thickness (mm)	$\Delta^{14}C$ (‰)	Estimated age (yr)
Needles	Recently abscised needles		86 (84–90)	0
Litter 1	Pine needles among green parts of mosses		95 (93–96)	1
Litter 2 (needles)	Structurally intact needles among dead mosses		103 (93–130)	3
Litter 2 (mosses)	Dead but structurally intact parts of mosses			
Fragmented litter	The upper half of the organic horizon	15 (7–25)	155 (125–181)	10
Humus 1	The upper two-thirds of the lower half of the organic horizon	10 (5–15)	211 (184–247)	16
Humus 2	Lower third of the lower half of the organic horizon	5 (3–10)	174 (144–230)	> 45
E-horizon	The pale eluvial horizon of the mineral soil			
B-horizon	The rust-red illuvial horizon of the mineral soil			

The thickness of the organic horizons is given as the mean value with the range in parentheses. The ^{14}C abundance is given as the mean $\Delta^{14}C$ of three samples per horizon (five for litter 2 (needles)) with the range in parentheses. The estimated age is based on the mean $\Delta^{14}C$ values, and needle abscission age (3 yr) is subtracted for all fractions.

cloned, and up to 25 clones were sequenced from each sample, resulting in 248 sequenced clones. PCR was carried out using the primers ITS1-F (Gardes & Bruns, 1993) and ITS4 (White *et al.*, 1990) with 55°C annealing temperature. PCR products were cloned with the TOPO TA Cloning Kit with the pCR®2.1-TOPO vector and One Shot TOP10 chemically competent *Escherichia coli* (Invitrogen, Carlsbad, CA, USA). Small amounts of bacteria were used directly for PCR, which was carried out us above, but with primers M13 Forward (GTAAAACGACGGCCAG) and M13 Reverse (CAGGAAACAGCTATGAC). The PCR products were purified with the QIAquick PCR purification kit (Qiagen) and sequenced with a CEQ 8000 Genetic Analysis System with the CEQ DTCS Quick Start Kit (Beckman Coulter, Fullerton, CA, USA).

Sequences from cloned fragments were compared with database sequences (NCBI) using the BLASTN algorithm (Altschul *et al.*, 1997), and database sequences with high homology to soil-derived sequences were selected as references. Sample sequences and database references were aligned using the CLUSTALW algorithm of MEGALIGN (DNAStar Inc., Madison, WI, USA) with the 'gap penalty' parameter reduced to 6 for pair-wise alignment and 10 for multiple alignment. Aligned sequences were compared for similarity by neighbour joining using PAUP* 4.0b10 (Swofford, 2002).

T-RFLP

In order to enable processing of a large set of samples, the cloning approach was combined with T-RFLP analysis (Dickie *et al.*, 2002). All samples (27 cores × 8 horizons = 216 samples) were used for T-RFLP analysis. In addition, to enable matching of T-RFLP patterns with sequences, all clones that were sequenced were also subjected to T-RFLP analysis. PCR was carried out as above, but the primers were labelled with WellRED fluorescent dyes, ITS1-F with D3-PA and ITS4 with D4-PA (Proligo, Boulder, CO, USA). The PCR amplicons were digested with restriction enzymes according to the manufacturers' instructions. The library clones were digested with *Alu*I (Amersham Biosciences, Freiburg, Germany), *Bsu*RI (Fermentas, Burlington, Canada), *Cfo*I (Promega), *Hin*fI (Promega) and *Taq*I (Fermentas), separately. PCR products from soil samples were digested with *Taq*I and *Cfo*I, separately, and, when further resolution was needed, with one or more of the other enzymes included in the library. The T-RFLP patterns were analysed with a Beckman Coulter CEQ 8000 Genetic Analysis System, using the CEQ DNA Size Standard Kit-600. Sample T-RFLP patterns were compared with the reference database constituted by T-RFLP patterns from the clones using the program TRAMP (Dickie *et al.*, 2002). To account for base calling during analysis on the Beckman Coulter CEQ 8000 Genetic Analysis System and for within-taxa variation of the ITS region, the threshold level for fragment size in TRAMP was set to ±2 bp.

Element and isotope analyses

Three samples from each of the upper six organic soil horizons (five samples of 'litter 2 (needles)') were analysed for ^{14}C content at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory (Gaudinski *et al.*, 2001). Radiocarbon data are expressed as $\Delta^{14}C$, defined as the difference in permille between the ^{14}C:^{12}C ratio in the sample and that of a universal standard (oxalic acid I, decay-corrected to 1950). The age of the organic matter was estimated by relating the ^{14}C content of the samples to published records of atmospheric ^{14}C concentrations from the last 50 yr (Levin & Kromer, 2004). Average residence time on the forest floor for the sampled organic matter was estimated by subtracting the age of recently abscised needles from the average age of the samples. All samples were analysed for N and C content and $\delta^{15}N$ using a C and N elemental analyser coupled on-line to an isotope ratio mass spectrometer (Model 20-20 IRMS; Europa Scientific Ltd, Crewe, UK) according to the procedures outlined in Ohlsson & Wallmark (1999). Results are expressed in $\delta^{15}N$ deviations from the standard atmospheric N_2: $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000$, where R denotes the ratio ^{15}N: ^{14}N.

Statistical analyses

The fungal community composition was related to spatial location of the samples and changes in elemental composition by canonical correspondence analysis (CCA) (terBraak, 1986) using CANOCO version 4.5 for Windows (Microcomputer Power, Ithaca, NY, USA). Two separate CCA tests were conducted, one with soil horizons included in the analysis as dummy variables, and one with changes in C:N ratio and ^{15}N natural abundance as explaining variables. In the second analysis, only litter 1 (L1), litter 2 (needles) (L2), fragmented litter (F) and humus 1 (H1) samples were included. Changes in C:N ratio and ^{15}N content throughout specific horizons were estimated by comparing data from the horizons immediately above and below a subsample. For L1 samples, values from recently abscised needles were subtracted from L2 (needles) values. For L2 (needles) samples, L1 values were subtracted from F values. For F samples, L2 values (average of needles and mosses) were subtracted from H1 values, and for H1 samples, values from F samples were subtracted from H2 values. Monte Carlo analysis with 1000 permutations was used to test for statistical significance of the environmental parameters in explaining the variation in community composition. Two Thelephoralean mat-forming taxa, which were highly dominating in two soil cores, were excluded from the multivariate analyses, as the samples from these cores drastically deviated from the others in community composition. Differences between soil horizons in C:N ratios and ^{15}N natural abundance were tested for statistical signifiance using analysis of variance (ANOVA) and Fisher's protected least significant difference test (PLSD).

Results

PCR products and T-RFLP community profiles were obtained from 204 of the 216 collected samples. Among the 248 sequenced clones, 100 different genotypes were identified, which all yielded unique combinations of restriction fragments, and 91 of these were encountered in the sample T-RFLP profiles. The nine taxa that were not retrieved probably had T-RFLP patterns with amplitudes below the 5% detection threshold.

The samples separated into two distinct groups according to the composition of the fungal community, with samples of intact needles containing different fungi from those in samples of fragmented litter, humus and mineral soil. Taxa recorded from intact needles were only detected sporadically in the more degraded material and vice versa. Dead parts of mosses contained an intermediate fungal community (Fig. 1). Within the deeper horizons, changes in the fungal community with soil depth could be observed, with some species found predominantly in the fragmented litter and humus and others being most common in the mineral soil. Canonical correspondence analysis attributed 10% of the total eigenvalue (the variation in species composition between samples) of the dataset to the vertical position of the samples. A Monte Carlo permutation test found the relationship between vertical position and community composition of samples to be highly significant ($P = 0.001$).

The 'early' fungal community, defined as the taxa occurring with a higher frequency in litter samples compared with older organic matter and mineral soil, was dominated by unidentified ascomycetes within the Helotiales and Dothideomycetes. Known saprotrophic fungi, such as *Mycena* and *Marasmius* spp., as well as plant parasitic *Lophodermium* spp., were also found in the surface litter. In the 'late' fungal community, defined as taxa occurring with a higher frequency in fragmented litter and humus than in litter, ectomycorrhizal basidiomycetes, predominantly from the genera *Cortinarius* and *Piloderma*, constituted a significant fraction. Ascomycetous *Capronia* spp. and *Rhizoscyphus ericae* (D.J. Read) W.Y. Zhuang & Korf, which both form mycorrhizal associations with ericaceous plants (Allen *et al.*, 2003), were also common. The 'late' fungal community contained taxa from within the *Helotiales*, but these were different taxa from the helotialean fungi detected in surface litter. Zygomycetes (*Mortierella* and *Umbelopsis* spp.) as well as a distinct group of unidentified ascomycetes (group G) also contributed to the 'late' community (Fig. 1).

The average ^{14}C abundance of the samples increased with depth and peaked in the upper humus layer, but was lower again in the bottom part of the humus, where the organic matter was assumed to be derived from plant matter produced before the rapid increase in atmospheric ^{14}C content associated with thermonuclear weapons testing during the 1960s. The average age of the C in the upper humus was estimated at 16 yr, whereas the fragmented litter, older litter and younger litter were estimated to have resided on the forest floor for on average 10, 3 and 1 yr, respectively (Fig. 2).

Throughout the upper litter layers, C:N ratios decreased threefold from recently shed litter down to the fragmented litter. From the fragmented litter down to the oldest humus, C:N ratios increased significantly by 22%, followed by a minor but significant decline in the mineral soil. In contrast, the ^{15}N abundance was constant in the litter layers, but increased with soil depth from the fragmented litter and downward (Fig. 2). In a canonical correspondence analysis, where the fungal communities in litter, fragmented litter and upper humus samples were related to changes in organic matter C:N ratio and ^{15}N content across the horizons, 7.3% of the total eigenvalue of the dataset was attributed to these two variables. A Monte Carlo permutation test found the relationship between the community composition of samples and changes in C:N ratio or ^{15}N abundance to be highly significant ($P = 0.001$). According to the multivariate analysis, fungal taxa known to be mycorrhizal were associated with positive changes in C:N ratio and large increases in ^{15}N natural abundance, whereas known saprotrophic fungi were associated with negative changes in C:N ratio and small changes in ^{15}N natural abundance (Figs 2, 3).

Discussion

We found a clear shift in fungal community composition between the surface litter (the L horizon) and the underlying F horizon, in which the litter had lost its structural integrity (Figs 1, 2). According to the ^{14}C enrichment of the organic matter, the oldest material in the L horizon had been residing on the forest floor for *c.* 3 yr, whereas the average age of the organic matter in the F layer was 10 yr. As the C in the uppermost part of the F layer is younger than the average age for the entire horizon, the shift in fungal community composition is likely to take place after *c.* 3–5 yr of decomposition.

Lophodermium species, which were frequently recorded in the L1 samples, are well-known needle endophytes, which have already colonized the needles when they enter the forest floor, but seem to be replaced by other fungi at a relatively early stage of decomposition (Fig. 1). The only saprotrophic basidiomycete that was recorded with a high frequency was a *Mycena* species. The most commonly detected taxa in the 'early' community were ascomycetes within the Helotiales and Dothideomycetes with uncertain taxonomy and ecology. However, as they rarely extended below the surface layer of the forest floor, which is devoid of roots, it seems likely that they are saprotrophs.

A large proportion of the 'late' community consists of ectomycorrhizal species within the genera *Cortinarius* and *Piloderma*. Whereas boreal trees generally form ectomycorrhizal associations, ericaceous dwarf-shrubs, such as *Calluna* and *Vaccinium* spp., form ericoid mycorrhizal associations (Smith & Read, 1997). In this study, *Rhizoscyphus ericae*, which is the

Sequence		Taxon	Litter 1	Litter 2 (needles)	Litter 2 (mosses)	Fragmented litter	Humus 1	Humus 2	E-horizon	B-horizon
test2.2b.2		Venturiaceae	0.69	0.85	0.70	0.11		0.04		0.05
test2.1b.11		Helotiales (Phialophora sp.)	0.38	0.41	0.48	0.07				
B1z 2.1		Helotiales group A	0.35	0.30	0.41	0.11	0.07	0.04		
A2z 3.6		Helotiales group A	0.12	0.48	0.59	0.11				
B2z 2.5	□	Mycena sp.	0.23	0.33	0.26	0.11			0.04	
A3x 2.1		Helotiales	0.27	0.22	0.22					
test2.1b.12		Helotiales	0.31	0.22	0.04	0.04				0.11
A1x 3.13		Dothideomycete group B	0.04	0.44	0.30			0.04		
test2.1b.9	□	Lophodermium pinastri	0.46	0.04			0.07	0.04	0.04	0.05
A1y 2.11		Cantharellales	0.15	0.15	0.04			0.04		0.05
test2.2b.11	□	Marasmius androsaceus	0.15	0.11						
A3y 2.14		Cantharellales	0.12	0.07						
A3x 2.3	□	Lophodermium sp.	0.19		0.04	0.04				
A2z 3.10		Helotiales	0.19		0.26				0.04	
test1.8a.27		Helotiales	0.19	0.04	0.30	0.04				0.11
test2.2b.3		Rhytismataceae group C	0.08	0.07	0.15	0.07				
C3x 1.9		Cantharellales	0.08	0.04	0.07					
A2z 5.3		Basidiomycete	0.04	0.07	0.07					
B1z 2.3		Basidiomycete		0.19	0.19	0.11	0.04	0.04		
A3y 3.11		Mollisia sp.	0.04	0.07	0.04	0.04			0.04	
A2x 2.8		Helotiales	0.04	0.04						
A2z 3.11		Capronia sp.	0.04	0.04	0.26					
C1z 3.5		Cantharellales	0.08	0.04	0.11					0.11
A3y 3.5		Helotiales group D	0.08	0.04	0.04	0.04	0.04	0.04		
test2.2b.15		Ascomycete	0.08		0.07			0.04		
A2z 3.5		Capronia sp.	0.12	0.07	0.37	0.07	0.04	0.04	0.08	0.11
A3x 2.2		Helotiales group A	0.04							
A3y 3.14		Venturia sp.	0.04		0.04					
-		Unknown		0.04						
B2z 2.4		Dothideomycete group B		0.04	0.04					
A2z 4.7		Rhytismataceae group C		0.04	0.11	0.07				
A2x 2.7		Cladosporium sp.		0.04		0.04			0.04	
A3y 3.4		Venturiaceae		0.04	0.04		0.04		0.04	
test2.3b.16	■	Sebacina sp.			0.11					
A3y 3.3		Helotiales group A			0.07					
A2x 2.6		Basidiomycete			0.04					
test2.1b.10		Helotiales group D		0.04	0.15	0.07	0.04			
C1z 3.1		Capronia sp.			0.07	0.04				
A1x 3.16	■	Cortinarius sp.			0.07	0.04				
C1z 3.6	□	Galerina atkinsoniana			0.04	0.04				
B3z 4.13		Basidiomycete			0.04	0.04				
test1.8a.2		Helotiales				0.04				
test1.8a.25		Tremellales				0.04				
B1z 4.9	■	Cortinarius sp.				0.04				
C2y 4.9	■	Cortinarius armeniacus				0.04				
A2z 5.8		Helotiales group E			0.04		0.04			
B1z 4.3		Unknown			0.07			0.04		
test1.8a.6		Capronia sp.							0.04	
test2.3b.13	■	Sebacina sp.			0.04			0.08		
A2z 5.4		Unknown						0.08		
A2z 3.3		Helotiales			0.04		0.04		0.04	
test1.8a.8		Morteriella sp.							0.08	
test2.3b.15	■	Dermocybe sp.	0.04		0.22	0.19				
test1.8a.5		Basidiomycete				0.04				0.05
A1y 2.14		Helotiales			0.19	0.07	0.04			
test2.1b.15		Basidiomycete			0.19	0.04	0.07			
C2y 8.3		Capronia sp.				0.04	0.04		0.04	
test2.3b.6		Capronia sp.			0.11	0.07				0.05
C1y 8.5		Ascomycete group G							0.08	0.05
A3y 3.7	■	Cortinarius sp.			0.15	0.11	0.04			
C1z 5.10		Ascomycete				0.04	0.07	0.04		
A1x 3.11		Atheliacceae			0.07	0.07	0.04		0.04	
A2x 5.1	■	Thelephorales				0.04	0.04	0.04	0.04	
C1y 8.4		Umbelopsis isabellina					0.07	0.04	0.04	
A3x 7.3		Umbelopsis sp.						0.04	0.12	
test2.3b.10		Basidiomycete			0.26	0.11	0.04		0.04	
A2z 5.13		Umbelopsis sp.				0.07	0.07		0.04	
C3z 4.1		Mollisia minutella	0.04	0.04	0.22	0.19	0.04	0.04	0.12	
A2z 4.9	▲	Rhizoscyphus ericae	0.04	0.04	0.11	0.19	0.07	0.08	0.08	
test1.5b.1		Morteriella sp.				0.04	0.07	0.08	0.04	
A1y 5.3	■	Hydnellum aurantiacum					0.07	0.08	0.08	
C3z 4.2	■	Dermocybe semisanguinea		0.04	0.15	0.07	0.22		0.04	
B3z 4.4	■	Cortinarius sp.	0.04	0.04	0.07	0.15	0.30	0.04		
A1y 5.4		Helotiales	0.12	0.11	0.37	0.41	0.22	0.15	0.12	
A3z 7.4	■	Phialocephala fortinii			0.15		0.11	0.19	0.04	
test1.5b.4		Unknown				0.04	0.07	0.15	0.08	
B1z 4.2	■	Cortinarius armillatus			0.04	0.15	0.11	0.08		0.05
test1.8a.1		Helotiales group F					0.04		0.04	0.32
A3z 6.7	■	Cortinarius sp.	0.04		0.04	0.15	0.15	0.12	0.08	
C2y 8.6		Umbelopsis sp.						0.04	0.20	0.16
test2.4b.10	■	Rozites caperatus				0.04	0.15	0.12	0.12	
A2z 3.1	▲	Capronia sp.	0.08	0.07	0.26	0.44	0.26	0.08	0.08	
test1.8a.4		Helotiales group F	0.04	0.04	0.04	0.04		0.08	0.20	0.42
C2y 8.2		Ascomycete group G					0.04	0.08	0.16	0.37
A2z 5.11		Ascomycete group G					0.04	0.19	0.16	0.26
A3z 7.2	■	Piloderma sp.		0.04	0.11		0.11	0.15	0.16	0.42
C1z 5.6		Helotiales group E		0.04	0.15	0.30	0.19	0.38	0.12	
A3z 6.8		Ascomycete group G			0.04	0.07	0.30	0.27	0.16	0.16
C1z 5.7		Unknown (Ascomycete group G)			0.07	0.33	0.56	0.31	0.04	
test2.4b.9	■	Piloderma reticulatum		0.04	0.30	0.59	0.56	0.46	0.12	0.05
A2z 4.6		Ascomycete group G		0.07	0.22	0.74	0.85	0.81	0.48	0.21

Fig. 1 Vertical distribution of fungal taxa in soil samples from a *Pinus sylvestris* forest. Columns represent different vertical horizons ranging from 'litter 1', which is needles at the surface, down to the 'B horizon' of the mineral soil. Cell figures are observation frequencies and the cells are coded as follows: light grey < 0.1; mid-grey 0.1–0.3; dark grey > 0.3. Open squares, known saprotrophic taxa; closed squares, known ectomycorrhizal taxa; triangles, known ericoid mycorrhizal taxa.

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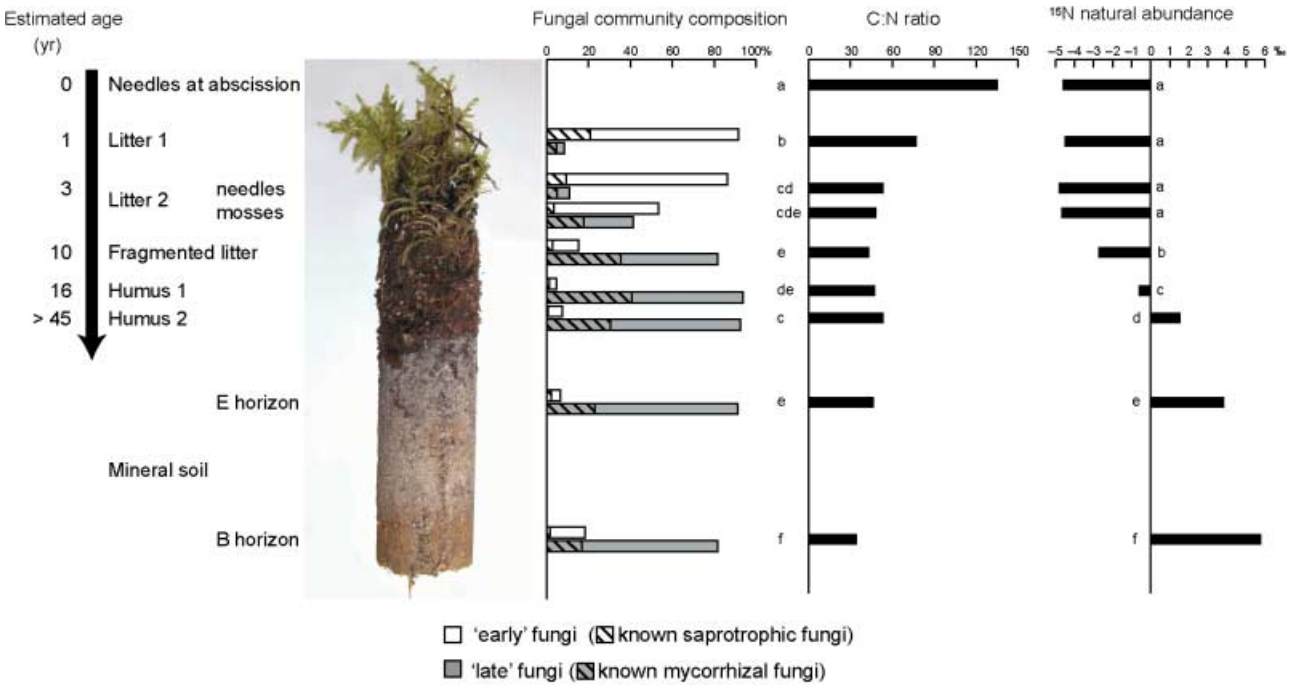

Fig. 2 Fungal community composition, carbon:nitrogen (C:N) ratio and ^{15}N natural abundance throughout the upper soil profile in a Scandinavian *Pinus sylvestris* forest. Different letters in the diagrams indicate statistically significant differences between horizons in C:N ratios and ^{15}N abundance, and the standard error of the mean was < 0.3‰ for ^{15}N natural abundance and < 3 for C:N ratio (n = 19–27, for recently abscised needles n = 3). The age of the organic matter is estimated from the average $\Delta^{14}C$ of three samples from each horizon (five samples of the litter 2 (needles) fraction) and needle abscission age (3 yr) is subtracted. Community composition data are expressed as the frequency of total observations. 'Early' fungi are defined as those occurring with a higher frequency in litter samples compared with older organic matter and mineral soil. 'Late' fungi are those occurring with a higher frequency in older organic matter.

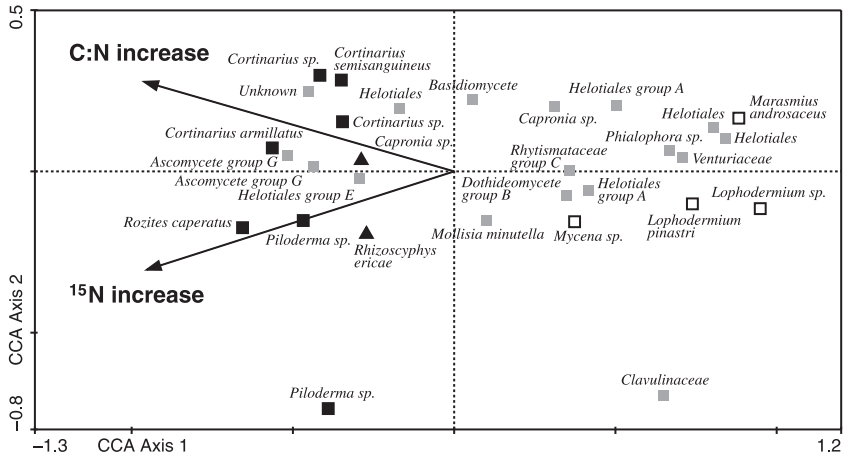

Fig. 3 Canonical correspondence analysis depicting the relation between fungal community composition and changes in carbon:nitrogen (C:N) ratio and ^{15}N natural abundance in a boreal forest soil. The arrows represent increasing C:N ratio or ^{15}N abundance with depth and age of the organic matter. Open squares, known saprotrophic taxa; closed squares, known ectomycorrhizal taxa; triangles, known ericoid mycorrhizal taxa; grey squares, taxa with uncertain ecology.

most thoroughly studied ericoid mycorrhizal fungus, was recorded less often than *Capronia* taxa, which have only recently been confirmed as ericoid mycorrhizal symbionts (Allen *et al.*, 2003). In contrast to the helotialean taxa found in the surface litter, helotialean taxa in the 'late' community generally show a high degree of sequence homology to taxa recorded from coniferous or ericoid roots, suggesting that the 'late' helotealean taxa are mycorrhizal or in other ways associated with roots (Fig. 4). The zygomycetous *Umbelopsis* species found in the 'late' community show a high degree of sequence homology to fungi isolated from surface-sterilized, healthy roots of *Pinus ponderosa*, suggesting that these fungi are root endophytes (Hoff *et al.*, 2004). The unknown ascomycetes (group G) that were detected in the deeper samples belong to

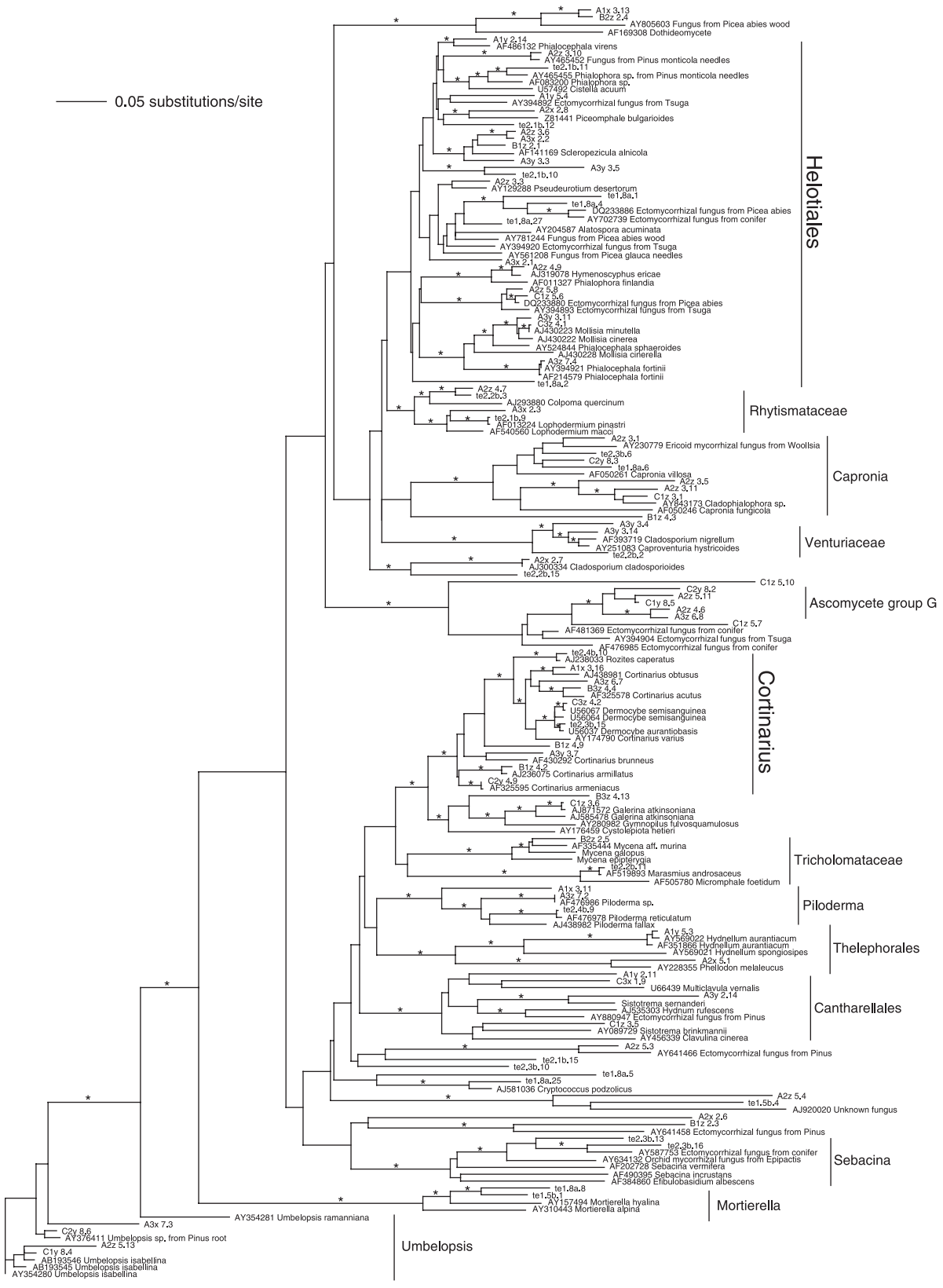

Fig. 4 Neighbour-joining tree of internal transcribed spacer (ITS) sequences obtained through PCR amplification of DNA extracted from soil from a *Pinus sylvestris* forest together with reference sequences from National Center for Biotechnology Information (NCBI). *, bootstrap values of 85 or higher.

a recently discovered lineage (Schadt *et al.*, 2003) that hitherto has been recorded only as environmental DNA and seems to be phylogenetically separate from other known ascomycetes. DNA from this group has frequently been obtained from ectomycorrhizal coniferous roots (e.g. Rosling *et al.*, 2003), suggesting that these fungi are also ectomycorrhizal or root endophytes (Fig. 4).

Based on mycelial ^{13}C abundance and drastically reduced mycelial production in plots with severed root connections, Wallander *et al.* (2001) claimed that most of the mycelium at the humus/mineral soil interface was mycorrhizal. This claim is now supported by our observation that the vast majority of DNA below the uppermost moss and litter layer may be assigned to known mycorrhizal fungi or otherwise root-associated fungi. In particular, the absence of DNA from nonhelotialean ascomycetes, including 'soil saprotrophs' such as *Trichoderma* or *Penicillium* spp., in the more decomposed samples is noteworthy. The scattered records of 'early' fungi in humus and mineral soil samples (Fig. 1) may be an artefact caused by litter fragments being pushed downwards by the sampling tool.

The finding that dead parts of mosses, in addition to the 'early' fungal community of the surrounding needles, also contain mycorrhizal fungi is in accordance with earlier observations that mycelium of *Cortinarius* spp. often extends from roots in deeper soil horizons to colonize dead moss tissues (Genney *et al.*, 2006). Mycorrhizal fungi have also been shown to efficiently colonize dead mosses in soil microcosms (Carleton & Read, 1991).

Samples colonized by the 'early' and 'late' fungal communities differed significantly with respect to their C and N dynamics, as shown by the canonical correspondence analysis (Fig. 3). Throughout the upper litter layers, where the 'early' and presumably saprotrophic fungal community is active, the C:N ratio decreases with time. Decreasing C:N ratios with progressing decomposition are commonly observed (Berg & McClaugherty, 2003) and indicate respiratory removal of C in combination with retention of N within the litter and its microbial community (Lindahl *et al.*, 2002). In contrast, the 'late' fungal community of mycorrhizal and other presumably root-dependent fungi, which was found in the fragmented litter and humus, was associated with increasing C:N ratios with organic matter age, indicating selective removal of N driven by root-derived C (Figs 2, 3). This presumption is corroborated by the simultaneous large increase in the stable isotope ^{15}N, which must be driven by fractionation against the heavier isotope during transfer of N from the soil through mycorrhizal fungi to their host plants (Högberg *et al.*, 1996; Högberg *et al.*, 1999; Hobbie & Colpaert, 2003). During this transfer, ectomycorrhizal fungi become *c.* 10‰ more enriched than their host plants, which is approximately the difference between the litter and the deeper soil layers observed here (Fig. 3). The significant enrichment in ^{15}N natural abundance in horizons dominated by ectomycorrhizal fungi thus indicates that plant N is mobilized via root-associated mycorrhizal fungi.

In a classic paper, Gadgil & Gadgil (1971) suggested that ectomycorrhizal and saprotrophic fungi compete with each other for N, and such competitive interactions could act to maintain the partitioning of the fungal community into two vertically separated and functionally distinct subcommunities, as observed in the present study. In soil microcosms we have previously observed antagonistic interactions between saprotrophic fungi and ectomycorrhizal fungi (Lindahl *et al.*, 1999), in which the outcome was strongly influenced by the amount of substrate available to the saprotrophs (Lindahl *et al.*, 2001). Saprotrophic fungi are more efficient than mycorrhizal fungi in colonizing and utilizing fresh, energy-rich litter (Colpaert & van Tichelen, 1996) and may thus be able to outcompete mycorrhizal fungi from the upper part of the forest floor. However, as the C:N ratio decreases and the substrate becomes depleted in available energy, the saprotrophs should become less competitive, which is consistent with the observed replacement of saprotrophs by mycorrhizal fungi that do not depend on litter-derived energy.

Lignin and humus are built up from polyphenolic structures that require oxidative enzymes for their degradation, and it has traditionally been assumed that saprotrophic 'white rot fungi' are the only organisms with the capacity to degrade these recalcitrant compounds fully (Rayner & Boddy, 1988). In this study, saprotrophic fungi appeared to be confined to the surface litter, and humus degradation and nutrient mobilization from deeper organic matter would therefore have to be ascribed to other functional groups of microorganisms such as bacteria or mycorrhizal fungi. Some bacteria have been shown to utilize products of lignin degradation (Merkens *et al.*, 2005) but reports of lignin and humus degradation by bacteria are scarce (Berg & McClaugherty, 2003). However, the capacity of ectomycorrhizal and ericoid mycorrhizal fungi to produce enzymes involved in degradation of organic matter and to mobilize organic forms of N is well documented from laboratory experiments (Abuzinadah *et al.*, 1986; Read & Perez-Moreno, 2003; Lindahl *et al.*, 2005). Melillo *et al.* (1989) emphasized the importance of well-degraded organic matter and humus in supplying plant N and suggested that labile C entering the soil via roots and associated mycorrhizal fungi may play an important role in driving mobilization of this N. The dominance of mycorrhizal fungi in well-degraded litter and humus observed in the present study supports the hypothesis that mycorrhizal fungi play a significant role in mobilizing N from well-decomposed organic matter in boreal forest soils. In less degraded litter the lack of mycorrhizal fungi implies that the major part of needle litter decomposition is carried out by saprotrophs on the surface of the forest floor.

Boreal forests have been identified as a major global C sink (Myneni *et al.*, 2001), and interactions between the cycles of C and N have been of paramount interest in discussions about predictions of effects of increasing atmospheric CO_2 concentration and N deposition on boreal and temperate forests (e.g.

Townsend *et al.*, 1996; Nadelhoffer *et al.*, 1999). Our observations highlight the need for further studies of C and N cycling in temperate forest ecosystems to acknowledge the spatial separation of major functional components of the microbial community.

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References

Abuzinadah RA, Finlay RD, Read DJ. 1986. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. II. Utilization of protein by mycorrhizal plants of *Pinus contorta*. *New Phytologist* **103**: 495–506.

Allen TR, Millar T, Berch SM, Berbee ML. 2003. Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots. *New Phytologist* **160**: 255–272.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**: 3389–3402.

Berg B, McClaugherty C. 2003. *Plant litter – decomposition, humus formation, carbon sequestration*. Berlin, Germany: Springer-Verlag.

terBraak CJF. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology* **67**: 1167–1179.

Carleton TJ, Read DJ. 1991. Ectomycorrhizas and nutrient transfer in conifer feather moss ecosystems. *Canadian Journal of Botany* **69**: 778–785.

Colpaert JV, van Tichelen KK. 1996. Decomposition, nitrogen and phosphorus mineralization from beech leaf litter colonized by ectomycorrhizal or litter decomposing basidiomycetes. *New Phytologist* **134**: 123–132.

Dickie IA, Xu B, Koide RT. 2002. Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytologist* **156**: 527–535.

Gadgil RL, Gadgil PD. 1971. Mycorrhiza and litter decomposition. *Nature* **233**: 133.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.

Gaudinski JB, Trumbore SE, Davidson EA, Cook AC, Markewitz D, Richter DD. 2001. The age of fine-root carbon in three forests of the eastern United States measured by radiocarbon. *Oecologia* **129**: 420–429.

Genney DR, Anderson IC, Alexander IJ. 2006. Fine-scale distribution of pine ectomycorrhizas and their extramatrical mycelium. *New Phytologist* **170**: 381–390.

Hibbett DS, Gilbert LB, Donoghue MJ. 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature* **407**: 506–508.

Hobbie EA, Colpaert JV. 2003. Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. *New Phytologist* **157**: 115–126.

Hoff A, Klopfenstein NB, McDonald GI, Tonn JR, Kim M-S, Zambino PJ, Hessburg PF, Rogers JD, Peever TL, Carris LM. 2004. Fungal endophytes in woody roots of Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*). *Forest Pathology* **34**: 255–271.

Högberg P, Högberg MN, Quist ME, Ekblad A, Näsholm T. 1999. Nitrogen isotope fractionation during nitrogen uptake by ectomycorrhizal and non-mycorrhizal *Pinus sylvestris*. *New Phytologist* **142**: 569–576.

Högberg P, Högbom L, Schinkel H, Högberg M, Johannisson C, Wallmark H. 1996. N-15 abundance of surface soils, roots and mycorrhizas in profiles of European forest soils. *Oecologia* **108**: 207–214.

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Löfvenius M, Read DJ. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* **411**: 789–792.

Landeweert R, Leeflang P, Kuyper TW, Hoffland E, Rosling A, Wernars K, Smit E. 2003. Molecular identification of ectomycorrhizal mycelium in soil horizons. *Applied and Environmental Microbiology* **69**: 327–333.

Levin I, Kromer B. 2004. The tropospheric $^{14}CO_2$ level in mid-latitudes of the Northern Hemisphere (1959–2003). *Radiocarbon* **46**: 1261–1272.

Lindahl BD, Finlay RD, Cairney JWG. 2005. Enzymatic activities of mycelia in mycorrhizal fungal communities. In: Dighton J, White JF, Oudemans P, eds. *The fungal community: its organization and role in the ecosystem, 3rd edn*. Boca Raton, FL, USA: CRC Press, 331–348.

Lindahl B, Stenlid J, Finlay R. 2001. Effects of resource availability on mycelial interactions and ^{32}P-transfer between a saprotrophic and an ectomycorrhizal fungus in soil microcosms. *FEMS Microbiology Ecology* **38**: 43–52.

Lindahl B, Stenlid J, Olsson S, Finlay R. 1999. Translocation of ^{32}P between interacting mycelia of a wood decomposing fungus and ectomycorrhizal fungi in microcosm systems. *New Phytologist* **144**: 183–193.

Lindahl B, Taylor AFS, Finlay RD. 2002. Defining nutritional constraints on carbon cycling in boreal forests – towards a less 'phytocentric' perspective. *Plant and Soil* **242**: 123–135.

Melillo JM, Aber JD, Linkins AE, Ricca A, Fry B, Nadelhoffer KJ. 1989. Carbon and nitrogen dynamics along the decay continuum – plant litter to soil organic-matter. *Plant and Soil* **115**: 189–198.

Merkens H, Beckers G, Wirtz A, Burkovski A. 2005. Vanillate metabolism in *Corynebacterium glutamicum*. *Current Microbiology* **51**: 59–65.

Myneni RB, Dong J, Tucker CJ, Kaufmann RK, Kauppi PE, Liski J, Zhou L, Alexeyev V, Hughes MK. 2001. A large carbon sink in the woody biomass of Northern forests. *Proceedings of the National Academy of Sciences, USA* **98**: 14784–14789.

Nadelhoffer KJ, Emmett BA, Gundersen P, Kjønaas OJ, Koopmans CJ, Schleppi P, Tietema A, Wright RF. 1999. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. *Nature* **398**: 145–148.

Nadelhoffer KJ, Fry B. 1988. Controls on natural N-15 and C-13 abundances in forest soil organic-matter. *Soil Science Society of America Journal* **52**: 1633–1640.

O'Brien HE, Parrent JL, Jackson JA, Moncalvo J-M, Vilgalys R. 2005. Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology* **71**: 5544–5550.

Ohlsson KEA, Wallmark PH. 1999. Novel calibration with correction for drift and non-linear response for continuous flow isotope ratio mass spectrometry applied to the determination of delta N-15, total nitrogen, delta C-13 and total carbon in biological material. *Analyst* **124**: 571–577.

Persson T, ed. 1980. *Structure and function of northern coniferous forests – an ecosystem study*. Ecological Bulletins 32. Stockholm, Sweden.

Rayner ADM, Boddy L. 1988. *Fungal Decomposition of Wood, its Biology and Ecology*. Chichester, UK: John Wiley & Sons Ltd.

Read DJ, Perez-Moreno J. 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist* **157**: 475–492.

Rosling A, Landeweert R, Lindahl BD, Larsson K-H, Kuyper TW, Taylor AFS, Finlay RD. 2003. Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. *New Phytologist* **159**: 775–783.

Schadt CW, Martin AP, Lipson DA, Schmidt SK. 2003. Seasonal dynamics of previously unknown fungal lineages in tundra soils. *Science* **301**: 1359–1361.

Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*. San Diego, CA, USA: Academic Press Inc.

Söderström BE, Bååth E. 1978. Soil microfungi in three Swedish coniferous forests. *Holarctic Ecology* **1**: 62–72.

Swofford DL. 2002. *PAUP*. phylogenetic analysis using parsimony (*and other methods), version 4*. Sunderland, MA, USA: Sinaur Associates.

Tedersoo L, Koljalg U, Hallenberg N, Larsson K-H. 2003. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. *New Phytologist* **159**: 153–165.

Townsend AR, Braswell BH, Holland EA, Penner JE. 1996. Spatial and temporal patterns in terrestrial carbon storage due to deposition of fossil fuel nitrogen. *Ecological Applications* **6**: 806–814.

Trumbore SE, Harden JW. 1997. Accumulation and turnover of carbon in organic and mineral soils of the BOREAS northern study area. *Journal of Geophysical Research – Atmospheres* **102**: 28817–28830.

Wallander H, Nilsson L-O, Hagerberg D, Bååth E. 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist* **151**: 753–760.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfland DH, Sninsky JJ, White TJ, eds. *PCR protocols: a guide to methods and applications*. San Diego, CA, USA: Academic Press, 315–322.

