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Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three?

Mona N. Högberg · Peter Högberg · David D. Myrold

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Abstract In Fennoscandian boreal forests, soil pH and N supply generally increase downhill as a result of water transport of base cations and N, respectively. Simultaneously, forest productivity increases, the understory changes from ericaceous dwarf shrubs to tall herbs; in the soil, fungi decrease whereas bacteria increase. The composition of the soil microbial community is mainly thought to be controlled by the pH and C-to-N ratio of the substrate. However, the latter also determines the N supply to plants, the plant community composition, and should also affect plant allocation of C below ground to roots and a major functional group of microbes, mycorrhizal fungi. We used phospholipid fatty acids (PLFAs) to analyze the potential importance of mycorrhizal fungi by comparing the microbial community composition in a tree-girdling experiment, where tree belowground C allocation was terminated, and in a long-term (34 years) N loading experiment, with the shifts across a natural pH and N supply gradient. Both tree girdling and N loading caused a decline of ca. 45% of the fungal biomarker PLFA 18:2ω6,9, suggesting a common mechanism, i.e., that N loading caused a decrease in the C supply to ectomycorrhizal fungi just as tree girdling did. The total abundance of bacterial PLFAs did not respond to tree girdling or to N loading, in which cases the pH (of the mor layer) did not change appreciably, but bacterial PLFAs increased considerably when pH increased across the natural gradient. Fungal biomass was high only in acid soil (pH < 4.1) with a high C-to-N ratio (>38). According to a principal component analysis, the soil C-to-N ratio was as good as predictor of microbial community structure as pH. Our study thus indicated the soil C-to-N ratio, and the response of trees to this ratio, as important factors that together with soil pH influence soil microbial community composition.

Keywords Fungi-to-bacteria ratio · Mycorrhizal fungi · Nitrogen fertilization · Tree belowground carbon allocation · Tree girdling

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M. N. Högberg (✉) · P. Högberg
Department of Forest Ecology,
Swedish University of Agricultural Sciences,
SLU, 901 83 Umeå, Sweden
e-mail: mona.n.hogberg@sek.slu.se

D. D. Myrold
Department of Crop and Soil Science,
Oregon State University, Corvallis, OR 97331, USA

Introduction

It is generally held that soil pH plays a dominant role in determining the composition of the general soil microbial community, e.g., that fungi are favored compared to bacteria at low pH, and vice versa (e.g., Alexander 1977). Also substrate quality, e.g., the C-to-N ratio, is known to be important in regulating the composition of the microbial community (e.g., Swift et al. 1979; Myrold 1999). This is because of the relationship between the C-to-N ratio of the decomposing microorganisms and their substrates, and the fact that fungi use substrates of wider C-to-N ratios than bacteria (Sterner and Elser 2002). In accordance with these assumptions, a drastic decline in the fungi-to-bacteria ratio was found when soil pH increased across a gradient in

boreal forest (Högberg et al. 2003), whereas the C-to-N ratio of the microbial biomass diminished along with the C-to-N ratio of the organic matter (Högberg et al. 2006a). The relative importance of pH versus the C-to-N ratio was not clarified; however, observations of no change in the fungi-to-bacteria ratio after increases in soil pH (0.5–2.5 units) in coniferous forest humus that had been limed, ash-treated, or alkaline-polluted (Bååth et al. 1992, 1995; Frostegård et al. 1993a), and the findings of lower fungi-to-bacteria ratio in soils of only slightly higher pH values (0.2 units) in natural forests (Pennanen et al. 1999) clearly indicate that additional factors other than soil pH are likely to be involved (e.g., Blagodatskaya and Anderson 1998; Bååth and Anderson 2003; Högberg et al. 2003).

Although most soil microorganisms are thought to be C limited, a significant group, the mycorrhizal fungi, does not experience C limitation unless their plant hosts decrease their belowground C allocation in response to high N availability and/or low light intensity, defoliation, and other stresses (Smith and Read 1997). Hence, we hypothesize the low fungi-to-bacteria ratio and fungal biomass at high soil pH, but low C-to-N ratio, may also be the result of lower host C supply to mycorrhizal fungi in response to high soil N availability (Smith and Read 1997; Wallenda and Kottke 1998; Högberg et al. 2003; Treseder 2004; Nilsson et al. 2005).

Studies of correlations among soil microbial community composition and soil chemical properties, such as pH and C-to-N ratio, cannot alone distinguish the roles played by pH and N supply. Here, we approached this problem by comparing the microbial community composition in natural boreal forest soils over wide ranges of soil pH and C-to-N ratio with that resulting from experimentally terminated C supply to ectomycorrhizal (ECM) fungi and from experimental N loading.

The objectives in this study were, thus, to examine links among soil chemistry, plants, and the soil microbial community, and to identify what chemical soil factor(s) are most important in determining microbial community structure. This was done by comparing three forest types along a natural gradient that encompasses most of the variation of soil pH and N supply previously found in Fennoscandian boreal forests (Dahl et al. 1967; Lahti and Väisänen 1987; Giesler et al. 1998), with contrasting microbial communities (Högberg et al. 2003), with: (1) a 4-year-old large-scale tree-girdling experiment, known to have experienced a significant decline in microbial biomass C (Högberg and Högberg 2002) and an elimination of ECM fungal sporocarp production (Högberg et al. 2001); and with (2) a N fertilization experiment with up to 34 years of continuous annual N loading (Högberg et al. 2006b).

Materials and methods

Natural boreal forest: soil pH and N supply gradients

The site is located northwest of the village of Betsele in northern Sweden (64°39′N, 18°30′E, 235 m altitude). We used three 90-m-long transects, 25–70 m apart, through a 130-year-old forest previously used as a model for landscape-scale variations in Fennoscandian boreal forests. Relations between plants and soil (Giesler et al. 1998; Högberg 2001; Nordin et al. 2001) and among plants, microbes, and soils (Högberg et al. 2003; 2006a) have been described in great detail. These transects encompass the variations in forest productivity, N supply, and soil pH encountered through Fennoscandian boreal forests, as they run from a dwarf shrub (DS), across a short herb (SH), to a tall herb (TH) forest type. The following nomenclature of species follows Tutin et al. (1964–1980). The DS forest is an open low productivity *Pinus sylvestris* forest; the field layer is dominated by ericaceous dwarf shrubs, e.g., *Vaccinium myrtillus*, *V. vitis-idaea*, and *Empetrum hermaphroditum*. The intermediate SH forest is a dense *Picea abies* forest; here, several short herbs are abundant, e.g., *Oxalis acetosella*, *Maianthemum bifolium*, and *Solidago virgaurea*. The highly productive TH forest, with the highest soil pH and N supply (Table 1), is dominated by *Picea abies* trees (up to 36 m in height); the field layer consists mainly of tall herbs, e.g., *Aconitum septentrionale*, *Actaea spicata*, and *Rubus idaeus*. Soils in the entire area are sandy till soils with many boulders, classified as Haplic Podzols (FAO 1988). The slope is 2%. Mean annual temperature and precipitation are 1°C and 570 mm, respectively. On average the site is covered by snow from late October until early May.

Tree-girdling experiment: direct manipulation of tree belowground C allocation

We used a large-scale tree-girdling experiment in a naturally regenerated 49- to 59-year-old Scots pine (*Pinus sylvestris* L.) forest at Åheden (64°14′N, 19°46′E, 175 m altitude), located 30–80 km from the other two sites. The forest is classified as a DS forest (with *V. vitis-idaea* and *Calluna vulgaris* as dominant field-layer species) and was subjected to tree girdling early (EG) or late (LG) in the summer of 2000. There were three replicate plots (30 × 30 m) of these treatments and the

Table 1 Selected soil chemical characteristics of the mor layer at the study sites including a natural gradient of three forest types (Betsele), the N fertilization experiment (Norrliden), and the belowground tree C flux experiment (Åheden)

Site	Forest type†	Treatment‡	pH§	C-to-N ratio	NH_4-N (μg g^{-1} o.m.)	NO_3-N (μg g^{-1} o.m.)
Betsele	**DS**	–	**4.0 (0.1) ab**	**38.1 (2.4) a**	**4.6 (1.8) ab**	**0.9 (0.5) ab**
	SH	–	4.6 (0.1) c	22.9 (1.1) c	5.2 (1.2) ab	0.7 (0.3) ab
	TH	–	5.3 (0.1) d	14.9 (0.3) d	15.9 (5.5) ab	3.4 (0.8) a
Norrliden	**DS**	**N0**	**4.1 (0.0) b**	**37.5 (1.2) a**	**0.5 (0.2) a**	**0.7 (0.2) ab**
	DS	N1	4.1 (0.0) b	31.1 (1.8) ab	39.9 (32.1) ab	1.5 (0.3) ab
	DS	N2	4.2 (0.0) b	27.7 (0.6) bc	88.4 (10.1) b	7.3 (1.8) a
	DS	N3	4.1 (0.0) b	27.2 (0.7) b	3.3 (2.0) ab	0.6 (0.1) ab
Åheden	**DS**	**C**	**3.7 (0.0) a**	**37.9 (1.3) a**	**2.2 (0.5) ab**	**0.0 (0.1) b**
	DS	EG	4.1 (0.0) b	38.1 (1.1) a	2.1 (0.5) ab	0.3 (0.1) ab
	DS	LG	4.1 (0.0) b	35.3 (1.6) a	5.6 (5.1) ab	0.0 (0.0) b
P-values			<0.001	<0.001	0.012	0.003

The natural un-treated dwarf shrub (DS) forest type plots at the three different locations are bold-faced. Different letters denote significant differences within columns (one-way ANOVA followed by Tukey's multiple comparison test ($df = 9, n = 30$). For ammonium and nitrate the normality test failed and non-parametric ANOVA was applied. *P*-values are presented at the bottom of the table. Mean ± 1SE. $N = 3, n = 3$

† *DS* Dwarf shrub, *SH* short herb, and *TH* tall herb forest type

‡ *N0* is the control and *N1, N2,* and *N3* denote increasing rates of N load. Note that N3 treatment was terminated in 1991. EG and LG stands for terminated tree belowground C flux early and late in the season, respectively, in the year of 2000

§ Soil:water ratio 1:3 (v/v)

control treatment. By girdling, the flow of photosynthate C to belowground parts and soil was terminated by cutting off the bark, including the phloem, of the stems at breast height (1.3 m). A subsequent 50% loss in soil respiration was interpreted as loss of activity by ECM roots and extramatrical mycelium (Högberg et al. 2001; Bhupinderpal-Singh et al. 2003). A parallel 32% decline in soil microbial biomass C of root-free soil was interpreted as a loss of mainly extramatrical ECM mycelium (Högberg and Högberg 2002). The climate is similar to that at Betsele.

N loading experiment: direct manipulation of soil C-to-N ratio (and indirectly of tree belowground C allocation?)

This experiment is in a 50-year-old low-productive Scots pine forest of DS forest type with *V. vitis-idaea* and *V. myrtillus* as dominant understory species. It is located on a gently (2–5%) sloping till soil at Norrliden (64°21′N, 19°45′E, 267 m altitude), about 65 km from Betsele and 30 km from Åheden. The climate is similar to that at the other two sites. NH_4NO_3 was applied annually to plots (30 × 30 m), at four rates, N0–N3, with three replicate plots per treatment. N0 is the untreated control, which only receives the background deposition of ca. 3 kg N ha^{-1} $year^{-1}$, N1 has received annual additions of ca. 34 kg N ha^{-1} $year^{-1}$ from 1971 to 2004, N2 twice the N dose of N1, and N3 received ca. 108 kg ha^{-1} $year^{-1}$ from 1971 to 1990, and is thus a high-N treatment recovering from the previous high N load (Högberg et al. 2006b). Further details about experimental design, soils, etc. are given by Tamm et al. (1999) and Högberg et al. (2006b).

Soil sampling

Sampling was performed on 18, 25, and 26 August 2004, at Betsele, Norrliden, and Åheden, respectively. We sampled the mor layer, which comprises the less decomposed F layer, where remnants of plants appear as fragments, and the H layer with amorphous humic material. The combined F + H horizons is the biologically most active compartment of these soils; 75% or more of the C added with litter is released as CO_2 from this horizon (e.g., Harrison et al. 2000; Persson et al. 2000). The uppermost part of the soil, which comprises litter and the bottom layer of mosses and/or lichens, was not sampled. We established a simple field laboratory at each site. In each case, i.e., at a location along transects at Betsele or a plot in the experiments, three samples of the organic mor layer (F + H horizons, approximately corresponding to Oe + Oa), were taken with a 0.15-m corer, and bulked together representing one replicate. We carefully cleaned the auger before sampling in each forest type and treatment, and used separate sieves for the further processing of samples. Wearing disposable gloves, we quickly and carefully sorted out roots and larger detrital materials (cones, twigs) before passing the soil through a sieve (5-mm

mesh), and weighed appropriate amounts of soil into closable plastic bags. A maximum period of 3 h at 11–14°C preceded storage on dry ice (−78°C) and thereafter in a freezer (−20°C) for soils analyzed for phospholipid fatty acids (PLFAs). Soil samples for chemical analysis were stored at 4°C (never frozen) and analyzed the day after sampling. At Betsele, sampling was done along the three separate transects, 25–70 m apart, each encompassing all three forest types. Thus, composited soil samples were collected in the DS forest type at three positions (at 0, 10, and 20 m distance) along the transects and within a perpendicular distance from each transect of less than 2.5 m. Composite samples were taken similarly in the SH (at 40, 50, 60 m distance) and TH forest types (at 80, 85, and 90 m distance). At Norrliden and at Åheden, three plots of each treatment were sampled. The central 400 and 100 m^2 of each plot were sampled randomly at Norrliden and Åheden, respectively. The mean values of each of three forest types along the three transects through each forest type (Betsele) and the mean values of three plots of each treatment (Norrliden and Åheden) were used in the statistical analysis ($N = 3$).

Soil chemistry

Soil pH was measured at a soil-to-water ratio of 1:3 (v/v). Extractable NH_4-N and NO_3-N of filtered soil extracts (20 g fresh soil in 50 ml of 2 M KCl, Munktell 00H equivalent to Whatman 42) were analyzed using a flow injection analyzer (FIAstar, FOSS Tecator Höganäs, Sweden). Dried soil samples (70°C, 84 h) were analyzed for total C and N using a CN analyzer coupled to a mass spectrometer (Ohlsson and Wallmark 1999). Organic matter (o.m.) content of the mor layer was defined as the loss of weight after ignition of dry soil (105°C, 24 h; 600°C, 4 h).

Microbial community composition

The composition of the microbial community was determined by analysis of group-specific PLFAs in soil (Tunlid and White 1992; Frostegård and Bååth 1996). Standard nomenclature is used to describe PLFAs (Tunlid and White 1992). Fatty acids are designated as the total number of C atoms:number of double bonds, followed by the position of the double bond from the methyl end of the molecule. The prefixes "a" and "i" refer to anteiso- and isobranching. A 10Me indicates a methyl group on the tenth C atom from the methyl end of the molecule. Cyclopropyl fatty acids are indicated by the prefix cy. Two PLFAs were used as indicators of fungal biomass, 18:2ω6,9 (Federle 1986; Frostegård and Bååth 1996; Olsson 1999) and 18:1ω9 (Bååth 2003). Twelve PLFAs represented bacterial biomass i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7, i17:0, a17:0, cy17:0, 17:0, 18:1ω7, and cy19:0 (Frostegård and Bååth 1996). Branched PLFAs have been found to be common in gram-positive bacteria, mono-unsaturated are reported to be indicative of gram-negative bacteria, and mid-chain branched fatty acids are found in large proportion in actinobacteria (O'Leary and Wilkinson 1988; Pinkart et al. 2002). The three most abundant terminally branched PLFAs (i.e., i15:0, i16:0, a17:0) were used as signature lipid biomarkers for gram-positive bacteria while the three most abundant PLFAs characteristic of gram-negative bacteria were: 16:1ω7, 18:1ω7, and cy19:0. PLFA 10Me16:0, 10Me17:0, and 10Me18:0 were used as biomarkers for actinobacteria. Both the neutral lipid fatty acid (NLFA) 16:1ω5 and the PLFA 16:1ω5 are suggested to be indicative of arbuscular mycorrhizal fungi (Olsson 1999). We use the ratio between NLFA and PLFA 16:1ω5 to distinguish between arbuscular mycorrhizal fungi and bacteria. The ratio is high in arbuscular fungi (1–200) and low in bacteria (<1) (Olsson 1999). Lipids were extracted from frozen soil equivalent to 0.15 g o.m. using the Bligh and Dyer (1959) method as modified by Frostegård et al. (1991, 1993b). Extracted lipids were separated on silica gel columns (Bond Elut LRC, SI 100 mg; Varian, Palo Alto, Calif.), and lipids were eluted in sequence with chloroform, acetone, and methanol. The chloroform fraction (containing neutral lipids, NLFAs) and the methanol fraction (containing PLFAs) were dried under N_2, dissolved, and subjected to mild methanolysis. The resulting fatty acid methyl esters were analysed on an Agilent 6890 GC (Agilent Technologies, Palo Alto, Calif.) equipped with a flame ionization detector. The column used was a 25-m-long Ultra 2 column (phenyl methyl siloxane; internal diameter, 0.2 mm; film thickness, 0.33 μm; Agilent Technologies, Palo Alto, Calif.). The identity of individual fatty acid methyl esters was based on comparison with spectra of authentic FAME standards (FAME 37-47885-U; Supelco, Bellefonte, Pa.), and by comparing the retention times for individual peaks with known peaks that had been identified by GC-MS. Methylnonadecanoic acid (Me19:0) was used as internal standard.

Statistics

We express data per g o.m. and mol% denotes the fraction of the total moles of PLFAs or NLFA. We used one-way ANOVA followed by Tukey's multiple comparison tests to evaluate differences between the DS forest types at Betsele, Norrliden, and Åheden. When

normality tests failed, one-way ANOVA on ranks was used. Using the same type of ANOVA and multiple comparison tests, with forest type at Betsele or treatments at the experimental sites as factors, we tested for differences in soil chemical characteristics and relative abundance of a few specific PLFA biomarkers. Means are reported ± 1 SE. In Figs. 3, 4, 5, we present the adjusted R^2 (R^2_{adj}), which is the explained variance. The explained variance is the explained variation adjusted for *df*. Pearson product moment correlation was used for test of correlations. Non-transformed data on concentrations of the individual PLFAs (expressed as mol%) were subjected to principal component analysis (PCA) because the $\log_{10}$ transformation that is often applied in this type of analysis did not change the outcome of the PCA analysis.

Results

Soil chemistry

Central to the comparisons made here, which aim at clarifying the underlying factors determining microbial community structure, is that the soil chemistry in the poor DS forest at Betsele is similar to that of the DS forest types at the two other experimental sites; i.e., the control plots at Åheden (C) and Norrliden (N0). We found only small differences among chemical variables (pH, C-to-N ratio, and inorganic N g^{-1} o.m.) among the DS forest types at the three sites (Table 1). In the mor layer soil of the DS forest at Åheden, the pH in the control plots was not different from the DS forest at Betsele, but 0.4 units lower than DS plots (N0) at Norrliden ($P < 0.05$). Neither C-to-N ratio, which ranged only between 37.5 and 38.1, nor the inorganic N levels, which ranged between 1.2 and 5.5 (μg N g^{-1} o.m.), differed among the DS forest types at the three sites.

Across the three natural forest productivity gradients at Betsele, i.e., from the low productivity DS, through the intermediate SH, to the highly productive TH forest type, soil pH increased from 4.0 to 5.3, inorganic N concentration from 5.5 to 19.3 μg g^{-1} o.m., and the soil C-to-N ratio declined from 38 to 15 (Table 1). These data are in general agreement with results of previous studies, except the data on pH_{H20}, which showed less variation than previously found in the soil solution using the soil centrifugation technique (e.g., Giesler et al. 1998).

At Norrliden, 34 years of N additions did not alter the pH of the mor layer (Table 1), which was roughly the same, although other studies have found small differences between control plots and fertilized plots (Högberg et al. 2006b). Soil inorganic N levels were considerably higher in N1 and N2 plots, whereas in the terminated N3 treatment, the inorganic N levels were not different from in the control plots (N0). The mean C-to-N ratio in the N1 plots was slightly lower than in N0 plots, and significantly lower in the N2 and N3 plots than in the N0 plots ($P < 0.001$).

At Åheden, tree girdling did not change any of the soil chemical properties studied other than pH, which increased significantly by 0.4 units. Soil C-to-N ratio, water content (not shown), and inorganic N content did not differ significantly, but there was a tendency of higher water content in the EG and LG plots and higher inorganic N level in one of the LG plots than in the control plots (Table 1).

Thus, the DS forest types at the three sites displayed no, or very small differences, in basic soil chemical characteristics such as pH and C-to-N ratio, in particular in comparison to the high pH and concentrations of extractable inorganic N, and low C-to-N ratio found in the TH forest type at Betsele, and the very high concentrations of extractable N and low C-to-N ratio found in N1–N2 plots at Norrliden.

Microbial community composition

When subjecting all the PLFA data to a PCA, the PC 1 and PC 2 components (*x*- and *y*-axis, respectively), together accounted for 68% of the variation (Fig. 1). Samples from the natural DS forest at Betsele, and control plots in the experimental DS forests at Norrliden and Åheden were found to the left along PC 1, which accounted for 41% of the variation. Thus, in agreement with the results from the chemical analysis, there were no major differences in microbial community composition among the DS forest types at the three sites (PC 1 scores subjected to one-way ANOVA on ranks $P > 0.05$). The TH and SH samples from Betsele were found to the right, clearly separated from all other sites (PC1 scores, one-way ANOVA, $P < 0.005$). Along PC 2, which encompassed 27% of the variation, the N fertilized plots at Norrliden (N1, N2, and N3) were different from all other plots including the natural SH and TH forests with high soil N levels at Betsele (PC 2 scores subjected to one-way ANOVA, $P < 0.001$), whereas N3 did not differ from N1 (PC 2 scores, one-way ANOVA, $P = 0.990$).

At Betsele, the fungal biomarker 18:2ω6,9 declined sharply from 12.6 in the poor DS to 1.6 mol% in the highly productive TH forest type (Fig. 2). The other fungal biomarker 18:1ω9 also decreased, but to a lesser extent from 13.9 to 7.1 mol%; the two fungal indicators were strongly correlated ($r = 0.923$, $P < 0.000$, $n = 9$).

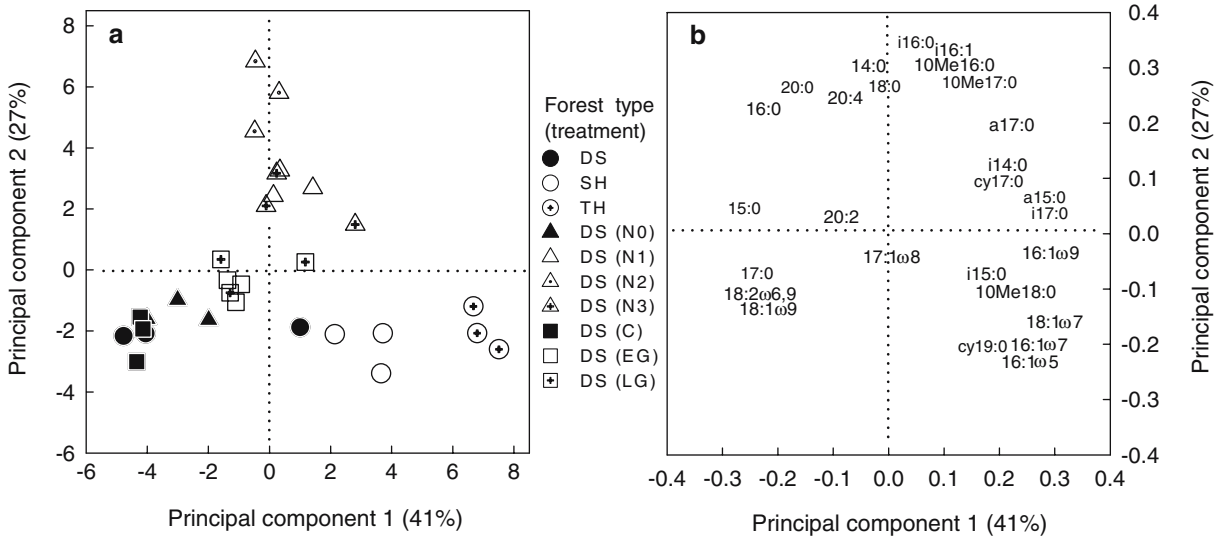

Fig. 1a, b Principal component analysis (PCA) of the signature lipid biomarkers (phospholipid fatty acids; PLFAs) used for examining the general soil microbial community composition in the mor layer in boreal coniferous forests in Sweden. The study encompassed three natural ecosystems commonly found in Fennoscandian boreal forests [dwarf-shrub (*DS*), short herb (*SH*), and tall herb (*TH*) forest type], a DS forest subjected to 34 years of N additions (*N0*, *N1*, *N2*, *N3*), and a second experimental DS forest, in which tree belowground C allocation was terminated by stem girdling early (*EG*) or late (*LG*) in the summer 2000. **a** PCA score plot of the two first principal components of the PLFA data set, providing a map of how the forest types or treatments relate to each other. **b** PCA loading plot of the first two principal components of the PLFA data set showing the individual PLFAs. The figure shows which PLFAs are influential (positioned far from plot origin), and how the variables are correlated. For example, the two fungal biomarkers (18:2ω6,9 and 18:1ω9) are grouped together (**b**) indicating they are positively correlated, and linked to DS, DS (N0), and DS (C) plots (**a**). PLFAs found in diagonally opposed quadrants are negatively correlated to each other

The sum of bacterial PLFAs significantly increased by 48% from the DS, SH, to the TH forest type (Fig. 2). Consequently, the fungi-to-bacteria ratio declined from 0.35 down to 0.03 when N supply and pH increased (Fig. 2). The biomarkers indicating gram-negative bacteria increased by 31% ($P = 0.005$), whereas those of gram-positive bacteria did not change significantly ($P = 0.054$). The PLFAs 18:1ω9, 18:2ω6,9, 20:2, and 20:4, indicative of eucaryotic organisms, were more common in the samples from the DS forest type (Fig. 1), whereas the other PLFAs, for example several iso- and anteiso-branched and cyclic PLFAs (i15:0, a15:0, i17:0, a17:0, cy17:0) as well as 10Me-branched (10Me16:0, 10Me17:0, 10Me18:0) and several mono-unsaturated ones (16:1ω5, 16:1ω7, 18:1ω7) were more common in the SH and TH forest types (Fig. 1). The NLFA 16:1ω5 constituted 1.4 ± 0.3, 4.0 ± 0.6, and 9.5 ± 1.1 mol% in the DS, SH and TH forest types, respectively. The PLFA 16:1ω5 constituted 2.6 ± 0.4, 3.9 ± 0.2, to 4.2 ± 0.1 mol% in the DS, SH, and TH forest types, respectively. Hence, the ratio between the NLFA and PLFA 16:1ω5 increases along the transects studied and indicates no or very low abundance of arbuscular fungi in the poor DS forest type (NLFA and PLFA 16:1ω5 ratio < 1), whereas in the rich SH, and especially in the highly productive TH forest type, the relative abundance of arbuscular fungi is high (NLFA and PLFA 16:1ω5 ratio > 1). The sum of the three PLFAs (10Me16:0, 10Me17:0, 10Me18:0), indicative of actinobacteria, increased significantly from 4.8 to 6.3 mol% ($P = 0.011$), i.e., 31% from the DS and SH forest type to the TH forest type.

Tree girdling at Åheden caused a reduction of about 45% in the PLFA 18:2ω6,9, but had no significant effect on 18:1ω9 (Fig. 2). Consequently, the fungal indicators PLFA 18:2ω6,9 and 18:1ω9 were not correlated ($r = 0.064$, $P = 0.870$, $n = 9$). NLFA 16:1ω5 constituted 0.9 ± 0.1, 1.7 ± 0.1 and 1.6 ± 0.1 mol% in control, EG and LG plots, respectively, whereas PLFA 16:1ω5 constituted 1.7 ± 0.1, 2.7 ± 0.2, and 2.7 ± 0.3 mol%, respectively. Thus the ratio between NLFA and PLFA 16:1ω5 was <0.6 in all treatments, suggesting that the increase in PLFA 16:1ω5 in tree-girdled plots was not caused by an increase in the abundance of arbuscular fungi, but rather by an increase in bacteria. At the time of sampling, 4 years after tree girdling, there was no longer a reduction in total microbial biomass as estimated by total PLFA (Fig. 2), but the fungi-to-bacteria ratio was significantly lower in girdled plots (Fig. 2). There was, however, a higher abundance of actinobacteria in girdled plots. The sum of the three PLFAs, indicative of actinobacteria, was 3.8, 6.1, and 5.8 mol%

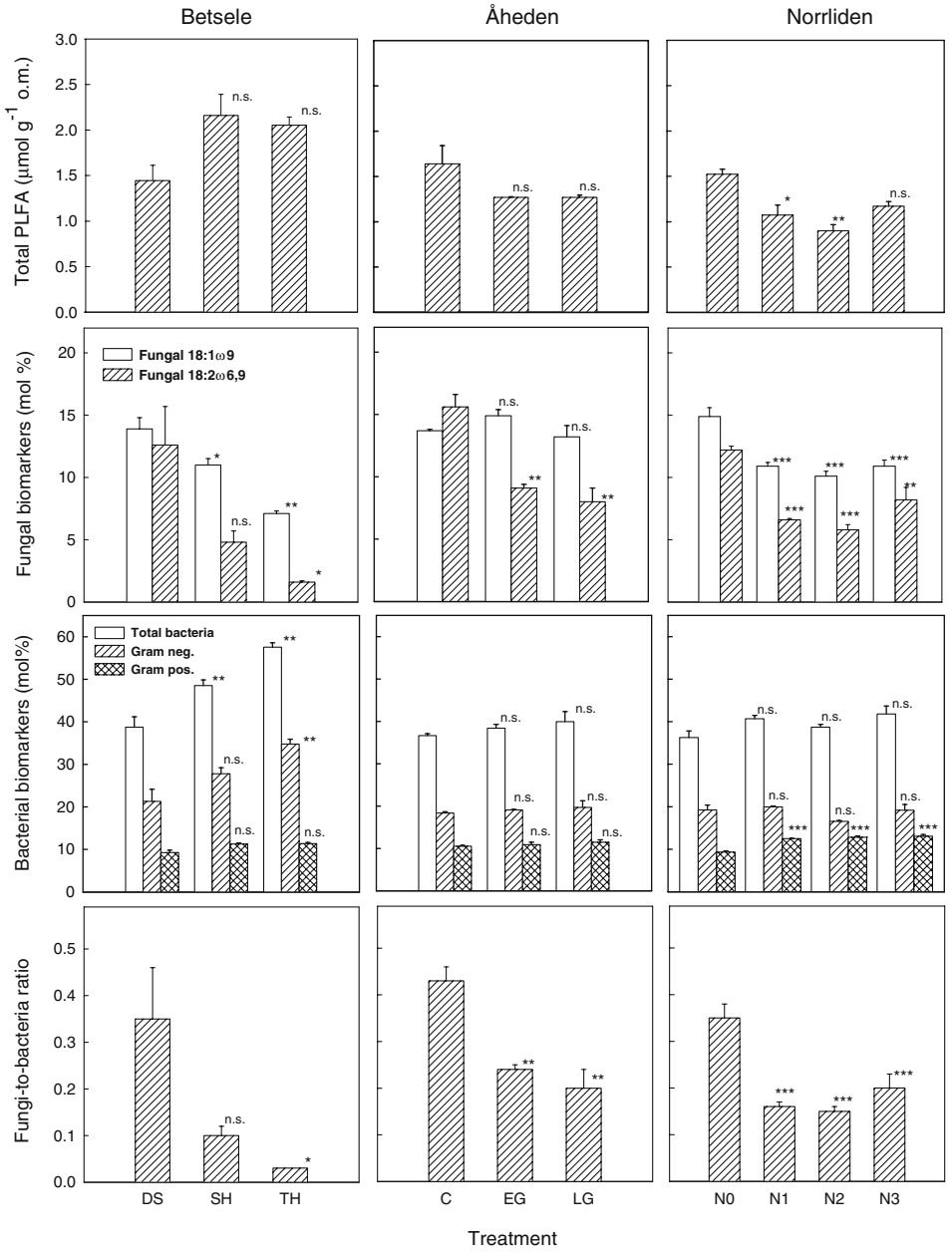

Fig. 2 Selected soil microbial community characteristics, based on signature lipid biomarkers (PLFA), at site Betsele, Norrliden and Åheden. Data are means ± 1SE, $N = 3$, $n = 3$. Relative to controls $P > 0.05$ (not significant; *n.s.*), $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

in the C, EG, and LG plots, respectively. This increase was significant ($P < 0.001$). The ratio of gram-negative to gram-positive bacteria did not change after tree girdling at Åheden (Fig. 2).

Long-term N loading at Norrliden caused a reduction in microbial biomass estimated by total PLFAs and by both fungal biomarkers 18:2ω6,9 and 18:1ω9, but increases in gram-positive bacteria, and hence decreases in the fungi-to-bacteria ratio (Fig. 2). Like at Betsele, the fungal indicators PLFA 18:2ω6,9 and 18:1ω9 were strongly correlated ($r = 0.921$, $P < 0.000$, $n = 12$). The abundance of NLFA 16:1ω5, indicative of arbuscular mycorrhizal fungi, did not differ significantly among N treatments and constituted 1.5 ± 0.1, 1.3 ± 0.2, 1.2 ± 0.2, and 1.7 ± 0.5 mol% in N0, N1, N2, and N3, respectively. The relative amount of PLFA 16:1ω5 was significantly lower in N1 (2.2 ± 0.1 mol%) and N2 (1.7 ± 0.1 mol%) plots compared with in N0 (2.8 ± 0.3 mol%) and N3 (2.7 ± 0.3 mol%) plots (one-way ANOVA, $P = 0.032$). The ratio between NLFA and PLFA 16:1ω5 was <0.7, hence the lower relative abundance of these two biomarkers in the N2 plots was more likely linked to bacteria than to arbuscular mycorrhizal fungi. Signature PLFAs indicative of actinobacteria, increased significantly ($P = 0.005$) and constituted 4.3, 7.6, 8.0, and 7.4 mol% in N0, N1, N2,

and N3 plots, respectively. There were tendencies that the terminated N-loading treatment, N3, was less different from the untreated control, N0, than the ongoing N treatments, N1 and N2, e.g., with regard to total PLFA and fungi-to-bacteria ratio. Interestingly, there was a strong negative relationship between the fungal biomarker 18:2ω6,9 and inorganic N (Fig. 3), according to which the abundance of this biomarker increased rapidly at concentrations below 10 μg inorganic N g^{-1} o.m., and along which the N3 plots grouped with N0 and N1 plots.

Thus, there were great similarities in microbial community composition in the DS forest types at the three different sites. Tree girdling caused a reduction of the PLFA 18:2ω6,9 in particular, while N loading caused reduction of both 18:2ω6,9 and 18:1ω9, in which cases their abundances increased sharply at <10 μg inorganic N g^{-1} o.m. Even larger reductions in these two fungal biomarkers occurred at Betsele, as soil C-to-N ratios declined, but extractable inorganic N and pH increased; the increase in fungal biomarkers again occurred below 10 μg inorganic N g^{-1} o.m. Plots of PLFA biomarkers against pH or log [(NH_4-N) + (NO_3-N)] produced remarkably similar positive relations for bacteria, but negative relations for fungi (Fig. 4). Higher values for actinobacterial biomarkers were found in the TH forest type at Betsele, the N-treated plots at Norrliden, and the girdled plots at Åheden.

Discussion

Both soil chemistry (Table 1) and microbial community composition (Fig. 1) were found to be similar in the DS forest types at the three sites Betsele, Åheden, and Norrliden. This is central for the comparisons between the natural gradients at Betsele and the experimental manipulations of the belowground C allocation

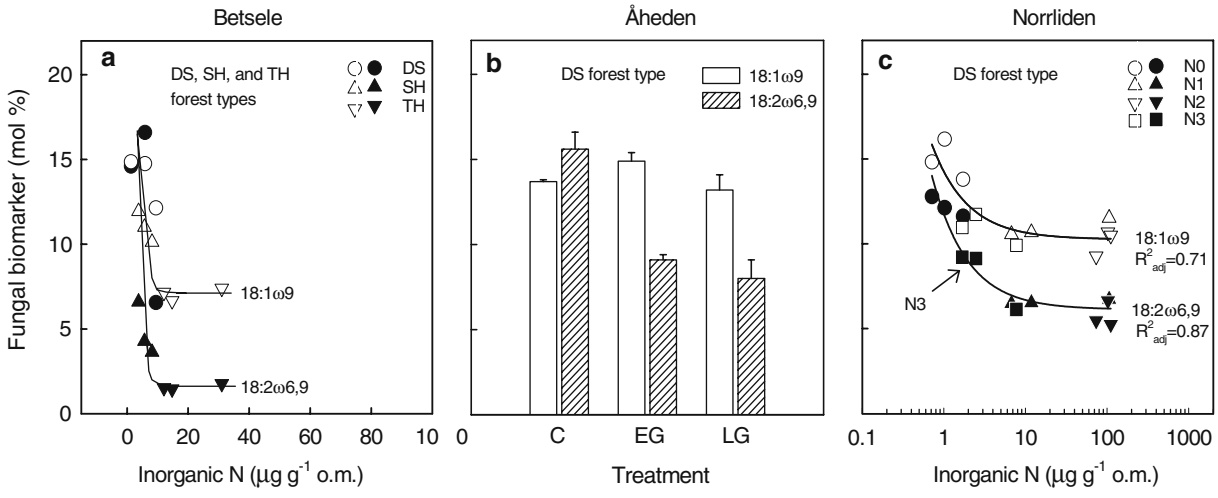

Fig. 3 **a** The effect of soil inorganic N concentration on mol% of fungal PLFA biomarkers at Betsele. **b** The effect of tree girdling, which terminates tree belowground C allocation, on fungal PLFA biomarkers at Åheden. **c** The effect of soil inorganic N concentration on fungal PLFA biomarkers at Norrliden. For abbreviations, see Fig. 1

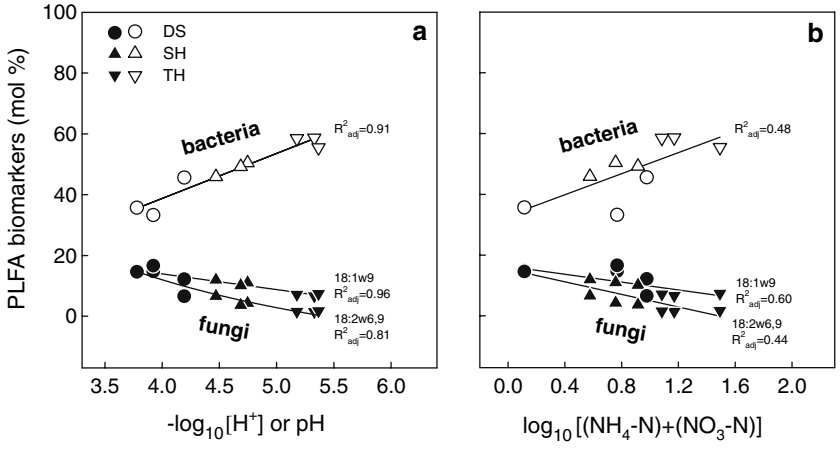

Fig. 4a, b Partitioning between major functional microbial groups in three forest types. The relationship between the bacterial and fungal signature lipid biomarkers and (**a**) $-\log_{10}$ [H+], i.e., soil pH, and (**b**) $\log_{10}$ [inorganic N]. Twelve PLFAs were used as biomarkers for bacteria and two PLFAs were used as indicators for fungi. For abbreviations, see Fig. 1

and soil N supply, as the two experiments were made at other sites, but as shown here, with untreated control plots similar to the low pH, high C-to-N ratio DS pine forest type at Betsele. In further support of our approach, we found that the structure of the microbial community in the DS and other forest types at Betsele appeared to be remarkably similar in 2004 (this study) and 1994 (Högberg et al. 2003), indicating stability of the microbial community structure under natural conditions.

Observations at Betsele are in line with the idea that the microbial community structure is determined by soil pH or C-to-N ratio (Figs. 4a, and 5; Table 1). However, we hypothesized (Högberg et al. 2003) that a decrease in the latter, and hence an increase in the supply of available N, should lead to a reduction in plant belowground C allocation. As shown here, in the tree-girdling experiment, termination of the belowground tree C supply leads to a considerable reduction of 18:2ω6,9 in particular. This is the fungal PLFA thought to be a good biomarker for ECM fungi (Frostegård et al. 1993a; Frostegård and Bååth 1996; Olsson 1999), the mycorrhizal symbionts of trees in this study, and for ericoid mycorrhizal fungi (Bååth et al. 2004), the mycorrhizal symbionts of ericaceous dwarf shrubs. Tree girdling specifically terminates the C supply to the ECM fungal symbionts of trees, as very clearly demonstrated by the almost total elimination of the production of sporocarps of ECM fungi on EG plots (Högberg et al. 2001). Meanwhile the effects of tree girdling on the ericoid mycorrhizal fungi symbiotic with the ericaceous dwarf shrubs are unclear. These dwarf shrubs had higher N% in their foliage after tree girdling (Bhupinderpal Singh et al. 2003), and seem to have responded with higher growth, but may have reduced their belowground C allocation, in response to the higher N supply when they were relieved from the competition from the trees. Hence, we suggest that the 45% reduction in PLFA 18:2ω6,9 mainly results from the total elimination of supply of photosynthate to ECM fungi. The fact, that 55% of 18:2ω6,9 still remains after tree girdling may suggest a considerable contribution from ericaceous fungi and/or saprotrophic fungi. At Betsele, PLFA 18:2ω6,9, declines to very low concentrations when pH increases and C-to-N ratio decreases; 18:1ω9 shows a smaller reduction. The fact that 18:2ω6,9 responds drastically to the tree girdling while 18:1ω9 does not, strongly suggests that the first is the better marker for ECM fungi. Surveys of fungal sporocarps revealed a rapid and almost total and elimination of ECM sporocarp production but no effect on saprophytic fungi (Högberg et al. 2001); a pattern which was evident also when this sampling was conducted in 2004.

N loading, a treatment generally held to reduce tree belowground C allocation (Cannell and Dewar 1994; Waring and Running 1998) also leads to a similar reduction in fungi (Figs. 2, 3, and 4); however, in this case both fungal biomarkers PLFAs 18:2ω6,9 and 18:1ω9 declined. The response of plant belowground C allocation to variations in nutrient supply is reversible, and it is possible there is an on-going recovery in the N3 plots, which were last fertilized with N 14 years before this study. It is, therefore, of considerable interest to note that the concentration of 18:2ω6,9 in soil from two of the N3 plots were intermediate to that in N0 and N1 plots, while the third N3 plot grouped with the N1 plots (Fig. 3). Other studies at Norrliden have

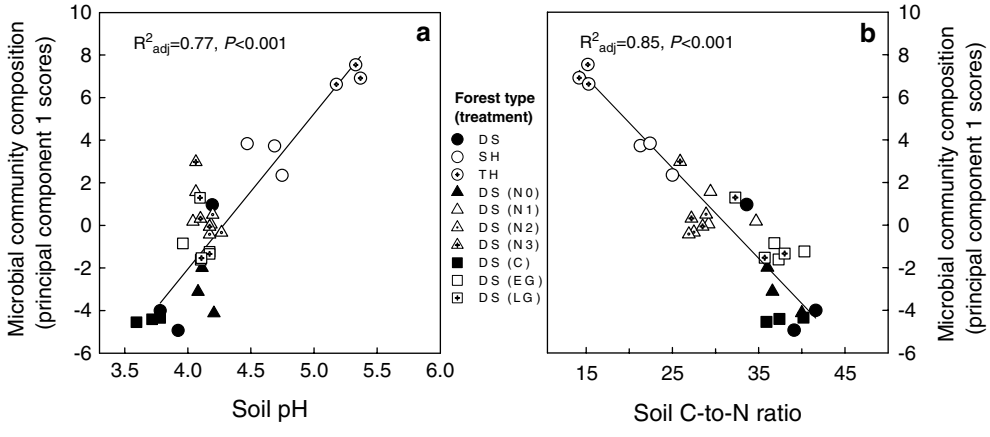

Fig. 5 Correlation between the soil microbial community structures and soil pH (**a**) or C-to-N ratio (**b**) in the natural DS, SH and TH forests at Betsele, the control (**c**) and tree-girdled (EG and LG) plots at Åheden, and the controls (N0) and N-loaded plots (N1, N2, N3) at Norrliden. Forests at Åheden and Norrliden are of DS type. For abbreviations, see Fig. 1

shown that soil pH and extractable inorganic N in the N3 plots are now similar to or approaching the values of N0 plots (Högberg et al. 2006b) and that the tree root uptake capacity for N, which is highest in the N-limited N0 plots, is becoming higher in N3 as compared to in the N1 and N2 plots (Quist et al. 1999).

Soil microbiologists in general assume that pH and/or C-to-N ratio of the substrate influence the general microbial community structure, but also think that these organisms are C limited. One group, the mycorrhizal fungi, is exceptional in this context, because of its immediate dependence on recent photosynthate, as demonstrated in the tree-girdling experiment, for example (Högberg et al. 2001; Högberg and Högberg 2002). Because of this strong dependence, it seems very likely that mycorrhizal fungi are sensitive to responses of their plant hosts to variations in N supply. The similarity in the reduction in PLFA 18:2ω6,9 after tree girdling and N loading suggests a response to reduction in photosynthate supply in both cases. Several recent studies point in the same direction, (e.g., Treseder 2004; Nilsson et al. 2005). Reduced plant C allocation induced by N additions has also been found to occur among arbuscular mycorrhizal fungi (e.g., Olsson et al. 2005a). Hobbie (2006) analyzed studies of mycorrhizal plants grown under controlled conditions in the laboratory, and found a close positive relationship between NPP allocated belowground and NPP allocated to mycorrhizal fungi, but a negative relation between NPP and the C cost for the mycorrhizal symbiosis; most of this variability was related to variations in the supply of nutrients. Accordingly, the high fungal biomass in the N-poor DS forest type may be linked to a high tree belowground C allocation, rather than the acidity of the soil. Likewise, the decline in fungal biomass when pH and N supply increases, e.g., at the Betsele gradients, may just as well reflect such a response to increasing N supply as a response to increasing pH (Fig. 5). A recent tree-girdling experiment demonstrated that the autotrophic respiration was lower in fertilized plots, despite a three-fold higher stem wood production (Olsson et al. 2005b). Our suggestion of a vital role played by changes in tree belowground C allocation in response to variations in N supply is also underpinned by the remarkable correlation between the microbial community composition and the C-to-N ratio (Fig. 5).

The disappearance of sporocarps of ECM fungi observed in areas of Central Europe subject to acid rain was initially interpreted as effects of acidifying S and N deposition directly on the soil biota (Arnolds 1991). In line with the evidence presented above, we suggest that it might also be attributed to a decline in tree belowground C allocation in response to the high N supply. The dramatic reductions in fungi as inorganic N supply increases (Fig. 3) strongly suggest that the coupling between N deposition and ECM fungi deserve further detailed studies.

Conclusions

The fungal and bacterial dominance at low and high soil pH, respectively, has been taken as evidence of a direct impact of soil acidity on microbial community structure. In Fennoscandian, and probably also in other boreal forests, soil pH is, however, in general negatively correlated with the C-to-N ratio of o.m., and the shifts in microbial community structure may also be related to direct influence of soil N availability, or to effects of N supply on plant belowground C allocation. Soil microbes are generally held to be C limited, but the possibility that soil N supply may directly regulate the C supply to mycorrhizal fungal symbionts, an important group in boreal forests, has not been widely recognized. The results of our study indicate that high N supply reduces the biomass of ECM fungi as much as tree girdling does, which specifically terminates the C supply to this group, and suggest an important indirect effect of soil chemistry on the microbial community composition. Laboratory studies have shown that changes in nutrient supply alter plant C allocation patterns profoundly, and are likely to exert major influences on soil microbial community structure and activity.

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