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Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs – a hypothesis based on field observations in boreal forest

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Summary

• Soil microorganisms are considered C-limited, while plant productivity is frequently N-limited. Large stores of organic C in boreal forest soils are attributed to negative effects of low temperature, soil acidity and plant residue recalcitrance upon microbial activity.

• We examined microbial activity, biomass and community composition along a natural 90-m-long soil N supply gradient, where plant species composition varies profoundly, forest productivity three-fold and soil pH by three units.

• There was, however, no significant variation in soil respiration in the field across the gradient. Neither did microbial biomass C determined by fumigation-extraction vary, while other estimates of activity and biomass showed a weak increase with increasing N supply and soil pH. Simultaneously, a phospholipid fatty acid attributed mainly to mycorrhizal fungi declined drastically, while bacterial biomass increased.

• We hypothesize that low N supply and plant productivity, and hence low litter C supply to saprotrophs is associated with a high plant C supply to mycorrhizal fungi, while the reverse occurs under high N supply. This should mean that effects of N availability on C supply to these functional groups of microbes acts in opposing directions.

Key words: boreal forest, carbon supply, microbial biomass, mycorrhizal fungi, nitrogen availability, saprotrophs, soil sampling, soil pH.

Introduction

Soil microorganisms are often said to be carbon-limited. This assumption, which is not valid for all organisms and conditions, is primarily based on frequent observations of a strongly enhanced respiratory activity after additions of labile C to the soil, e.g. sugars (Anderson & Domsch, 1978) or more complex C sources (Wu *et al.*, 1993). By contrast, plant photosynthesis and production is often nitrogen-limited, especially outside the tropics (Tamm, 1991). The C available to soil microorganisms is ultimately derived from plant photosynthesis and it could therefore be expected that processes or factors that determine the availability of soil C have the proximal control on microbial activity. Evidence of a coupling between plant production and the microbial biomass in the forest floor was found by Zak *et al.* (1994) in a study along a continent-wide gradient in North America, where a positive linear relationship between above-ground primary production and microbial biomass C was found. Myrold (1987) and Myrold *et al.* (1989) reported positive correlations between net primary production and microbial biomass C and N in mineral soil. Consequently, limitations on plant C assimilation may indirectly impose limitations on soil microorganisms. Hence, conditions favourable to plant production should also imply a high supply of C to saprotrophs via plant litter.

Mycorrhizal fungi, on the other hand, should be viewed as integral parts of the root systems of the autotrophic plants with regard to their main C supply. Hence, they may experience the same decrease in allocation of C from the shoot as the plant root systems do when the plant acclimatises to increased nutrient supply (Nylund, 1988; Wallander & Nylund, 1992). Based on field root exclosure experiments Gadgil & Gadgil (1975) showed that litter decomposition was retarded in the presence of ectomycorrhizal fungi. These were thought to be superior competitors

for the limiting nutrient, nitrogen, because of their high direct supply of labile C from the plants. Alternatively, ectomycorrhizal fungi could have exuded compounds harmful to the saprotrophs.

Recent laboratory microcosm experiments have, indeed, revealed combative interactions between mycorrhizal and saprophytic fungi. In these experiments, the organism with the larger supply of C succeeded in competition for a nutrient, phosphorus (Lindahl *et al.*, 2001; Lindahl *et al.*, 2002). However, mycorrhizal roots produce large quantities of dissolved organic C, which may act as a C source for other soil microorganisms (e.g. Högberg & Högberg, 2002). Thus, there is also evidence of positive effects of mycorrhizal roots on other soil microorganisms (Garbaye, 1994; Timonen *et al.*, 1998). The relations between plant growth, mycorrhizal fungi, other soil microorganisms and soil chemical properties may therefore be very complex. Ecologists assume important roles in the C cycle for both saprotrophs and mycorrhizal fungi, but the factors determining the interrelationships between the two functional groups remain unclear.

In Fennoscandian boreal forest, plant species composition and productivity commonly increases profoundly down hillslopes (Högberg, 2001). Leaching of N from upslope forest sites is minor and can not alone explain the observed higher N supply in groundwater discharge areas in toe-slope positions of unpolluted boreal forests (Högberg, 2001). Discharge areas are flushed with groundwater rich in base cations. It is likely that this has a decisive influence on the soil microbial community, that is its size, structure and activity, and thereby indirectly also on soil N dynamics (Högberg, 2001). In a recharge area the soil water had a relatively high concentration of amino acid N, whereas the soil water of a contiguous discharge area down-slope had much of its plant available N in the forms of NH_4^+ and NO_3^- (Nordin *et al.*, 2001). It is thus likely that soil N dynamics of discharge areas is distinctly different from that of soils of recharge areas, and that the soil N supply is under strong microbial control.

Effects of hill-slope hydrochemistry on soil microorganisms could relate to a number of factors. There could be influences of soil pH and other chemical characteristics directly on the soil microorganisms. This could affect the contribution by different functional groups, for example the ratio fungi : bacteria (Frostegård & Bååth, 1996; Blagodatskaya & Anderson, 1998) or the size of the total microbial community (Wardle, 1992; Anderson & Domsch, 1993; Anderson & Joergensen, 1997). There could also be soil effects on plant productivity and community composition and thereby the quantity and quality (chemical composition) of the organic matter produced by plants, which in turn is the substrate for microbial decomposers. Moreover, as soil factors affect the plant community, they also determine the identity, biomass and activity of the community of mycorrhizal fungi (Read, 1991). This latter suggestion is especially interesting in view of the observation that respiration by roots and their ectomycorrhizal fungi is of the same order as heterotrophic respiration (Högberg *et al.*, 2001; Bhupinderpal-Singh *et al.*, 2003). At the same time the extramatrical mycelium of ectomycorrhizal fungi may account for more than one third of the soil microbial biomass (Högberg & Högberg, 2002) in strongly N-limited boreal forests.

Here, we focus on estimates of microbial biomass, activity and community structure along a previously studied hydrochemical and plant productivity gradient at Betsele, northern Sweden (Högberg *et al.*, 1990; Giesler *et al.*, 1998; Nordin *et al.*, 2001). This gradient represents much of the variability in the boreal forest landscape in terms of variations in soil pH, C : N ratio, plant available N sources and plant productivity. Our study comprises data gathered over several years, which are used to present a general hypothesis about the balance between mycorrhizal fungi and other soil microorganisms. We initially hypothesized that: first total soil microbial biomass-C and -N increases (both per g o.m. and m^2) when plant productivity increases, second a higher nutrient supply and a higher soil pH should result in a higher turnover of organic matter, that is in higher microbial activity per g organic matter (which we regard as an index of substrate quality), third the type of mycorrhiza present should vary along with changes in plant community composition (Read, 1991), while fourth the biomass of mycorrhizal fungi would decrease as soil N availability increases (e.g. Nylund, 1988).

Materials and Methods

Field site

The 90-m-long gradient is located at the bottom of the Umeå River valley at Betsele, northern Sweden (64°39′-N, 18°30′-E, 235 m above sea level). The gradient has a rather constant slope of 2% and the mean annual temperature and precipitation are 1.0°C and 570 mm, respectively. On average the area is covered by snow from late October to early May. The plant-soil relations have been described in detail previously (Högberg *et al.*, 1990; Giesler *et al.*, 1998; Nordin *et al.*, 2001).

The upper end of the gradient (0 m) is located in an extensive recharge area with a *c.* 130-yr-old open *Pinus silvestris* L. forest and towards the discharge area there is an increasingly dense *Picea abies* (L.) Karst. forest of similar age (Fig. 1). Around the low end of the gradient (90 m) windfalls have opened up the forest and the visibly gradual increase in productivity cannot be accurately quantified. The Pinus forest (here represented by 0–40 m) has an average productivity (as estimated by two independent methods applied to areas > 1 ha) of 2.9 m^3 stem wood ha^{-1} yr^{-1}, while the Picea forest (50–90 m) has an average productivity of 8.0 m^3 stem wood ha^{-1} yr^{-1} (P. Högberg and L. Kinell, unpubl. data). The potential productivity should be even higher at the very end of the gradient (90 m). At the poor upper portion (0–40 m), the field layer is dominated by ericaceous dwarf shrubs. After about 40 m, there is an increasing amount of short herbs in the direction to the rich end, where tall herbs are dominant,

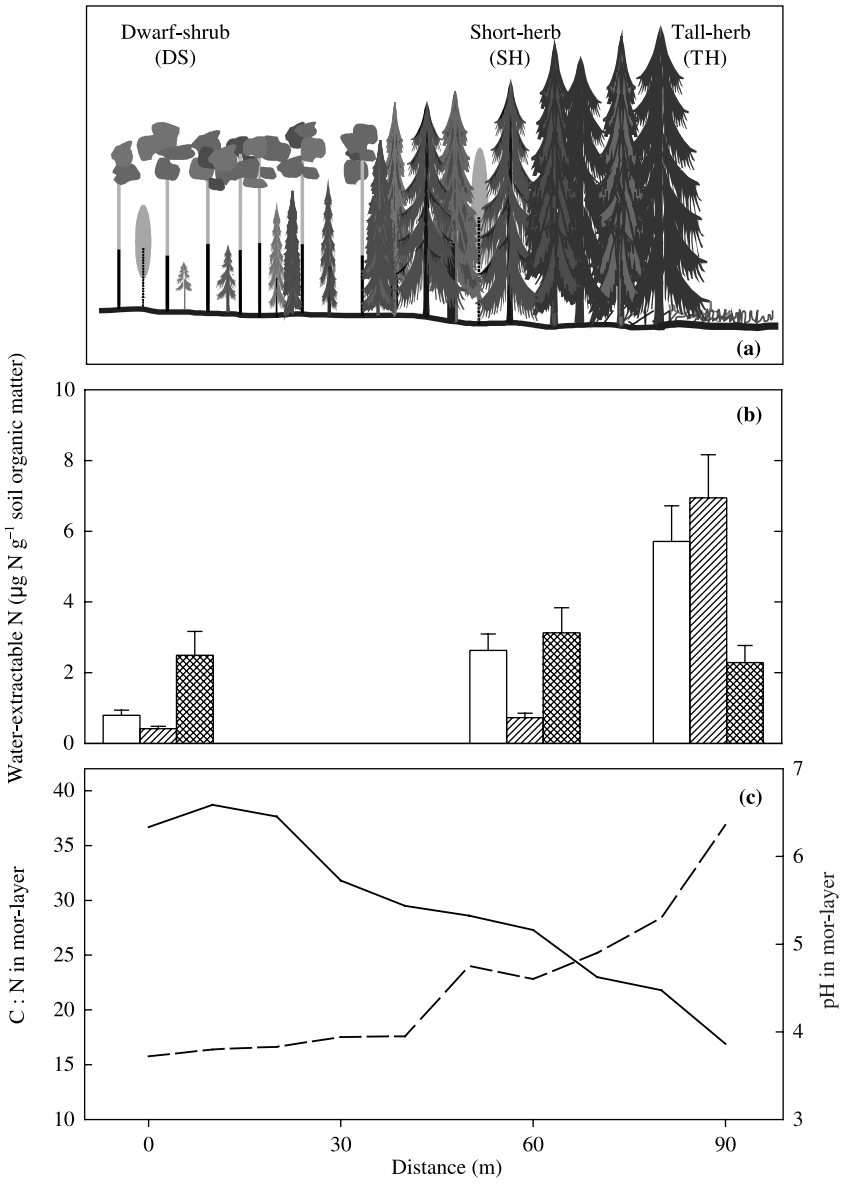


Fig. 1 The Betsele plant productivity gradient. (a) forest types: dwarf-shrub (DS) between 0 and 40 m, short-herb (SH) between 50 and 80 m and tall-herb (TH) at 90 m. (b) water-extractable N forms: ammonium (open bars), nitrate (hatched bars) and amino acids (crossed bars). (c) the C : N ratio of the mor-soil (solid line) and pH in the soil solution (dashed line). Data from Giesler *et al*. (1998) and Nordin *et al*. (2001).

in particular in the glade. Three forest types have thus been defined along the gradient (Giesler *et al*., 1998): a dwarf-shrub forest-type (DS) between 0 and 40 m, a short-herb forest-type (SH) between 50 and 80 m, and a tall-herb forest-type around 90 m (Fig. 1).

The soils are uniform sandy till soils with many boulders and are classified as Haplic Podzols (FAO, 1988). The mor-layer (O-horizon) was *c*. 7 cm thick throughout the gradient. At the upper part of the gradient the H (humus)-horizon was very thin, but increased in thickness successively along the gradient, and at the lower end the H horizon dominated. The thickness of the F (fermentation)-horizon showed the reverse pattern, dominating the O (organic)-horizon in the upper part but decreased successively in thickness along the gradient. The mor layer soils between 70 and 90 m were named F + H soil, since it was difficult to distinguish between the two horizons. The organic matter content in the combined F and H horizon were 91, 71 and 50% for the DS, SH and TH forest types, respectively.

In the spring, the discharge area at 90 m becomes flooded during up to a few weeks by groundwater rich in base cations, e.g. Ca and Mg. During these short floods, the groundwater level may rise and fall several decimetres in a day. Seasonal variations in soil water content of the mor-layer have been detected. The average soil water content g g^{-1} d. wt in 1994 and 1997 was 282 ± 65%. Apart from the discharge events in the TH forest type, differences in soil moisture of these highly organic soils are small along the gradient. Soil samples were not taken, nor was soil respiratory activity measured, during discharge events. The mean soil temperatures (n = 3) at the boundary between the mor-layer and the mineral soil on May 31, June 25 and August 9 in 2002 were 10.1 ± 2.7°C,

10.2 ± 2.4°C and 9.9 ± 2.2°C for the DS, SH and TH forest types, respectively. A significant difference between the dates was found ($P = 0.003$), but there was no significant difference in soil temperature across the gradient ($P = 0.874$).

The C : N ratio of the mor-layer soil decreased from 39 in the DS type at 0 m to 17 in the TH type at 90 m, while the pH in the soil solution increased 2.9 pH units (Fig. 1). The total N pool in the mor-layer, in kg per hectare, increased *c.* 3.6 times from the DS to the TH forest type, NH_4^+ in the soil solution increased from 0 to 80 m, but from 70 to 90 m NO_3^- became the most abundant inorganic N form in the soil solution (Giesler *et al.*, 1998). However, the concentration of amino acids exceeded that of inorganic N in the DS type, was equal to inorganic N in the SH type and lower than inorganic N in the TH type (Nordin *et al.*, 2001) (Fig. 1). The sum of water-extractable inorganic and organic N was 90, 122 and 479 g ha^{-1} in the DS, SH and TH types, respectively.

Soil sampling

Soil samples were taken on June 8, 1994 for extraction of ATP and phospholipid fatty acids (PLFAs), thymidine incorporation studies, acridine orange direct counts (AODC) for estimating bacteria and bacterial pH tolerance measurements. Monoliths of the entire organic mor-layer (0.16 m^2) were taken at every 10 m along the gradient. Soil samples from 0 to 60 m were divided into F and H horizons but at 70, 80 and 90 m no visual separation of the two horizons was possible so combined FH horizons were used. Additional F + H cores (diam. = 5 cm) were taken on July 8, 1994, every 5 m for measurements of basal respiration and SIR (substrate induced respiration). The soils were sieved (5 mm) and kept at 4°C for 2 wk. Microbial biomass-C and -N were determined by the fumigation extraction technique (FE) on June 17, August 4 and September 27 1997. For this, mor-layer soil (F + H) was sampled with an 10-cm diameter auger. Five replicate samples were taken within a 100-m^2 area at 0, 60 and 90 m. After removing roots > 2 mm, the samples were placed in plastic bags under the mor-layer for < 30 h at 7.2–12.9°C depending on sampling date. Organic matter content (o.m.) was estimated as weight loss after 4 h of ignition at 600°C on subsamples of dried soil (105°C, 24 h).

Root sampling

On 10 October 2002, we sampled soil to determine the biomass of living fine-roots (< 2 mm diameter) in the mor-layer. Ten cores (10 cm diam.) were taken at 0, 60 and 90 m. The intact cores were stored at −18°C and then thawed at +4°C before processing. Living fine-roots were separated into three groups consisting of roots of *Pinus sylvestris*, *Picea abies* and other species. The roots were dried (85°C, 24 h). For detailed description of the characters of living roots see Majdi & Persson (1995 and references therein).

Analysis of soil samples

Microbial biomass ATP was determined by using firefly luciferin-luciferase (Arnebrant & Bååth, 1991) on samples incubated at 25°C before extraction of ATP. SIR, the respiratory response was measured after glucose addition (Nordgren *et al.*, 1988). The total amount of PLFA (totPLFA) was determined following Frostegård *et al.* (1993). Microbial biomass-C and -N was estimated by the chloroform FE (fumigation extraction) technique (Brookes *et al.*, 1985; Vance *et al.*, 1987; Högberg & Högberg, 2002). Bacteria were counted microscopically after AO staining (Söderberg & Bååth, 1998). For conversion to microbial biomass C the factors used were: 1 mg biomass-C corresponded to 3.2 μg ATP (Arnebrant & Bååth, 1991), 340 nmol PFLA (Frostegård *et al.*, 1991) and 50 μg CO_2 h^{-1} SIR (Anderson & Domsch, 1978). Fumigation extraction followed Högberg & Högberg (2002), except that interference by nitrate (which was considered negligible) was not eliminated (Wyland *et al.*, 1994). For conversion to microbial biomass-C and -N, the correction factors used were $k_{EC} = 0.4$ and $k_{EN} = 0.4$ (Martikainen & Palojärvi, 1990).

Microbial community structure The composition of the microbial community was determined by analysis of group-specific PFLAs in soil (Tunlid & White, 1992; Frostegård & Bååth, 1996). Specifically, the PLFA 18 : 2ω6, 9 was used as an indicator of fungal biomass (Federle, 1986), and the sum of the bacterial PFLAs as an indicator of the bacterial biomass. Lipids were extracted from fresh soil (1 g) using the Bligh & Dyer (1959) extraction technique as modified by Frostegård *et al.* (1993). Polar lipids (phospholipids) were separated on silica acid columns, dried under N_2 air and subjected to mild alkaline methanolysis. The resulting fatty acid methyl esters were analysed on a gas chromatograph with a flame ionisation detector. Methyl nonadecanoate fatty acid was used as the internal standard.

Microbial activity The total microbial activity in sieved soil samples was estimated as basal respiration at 20°C (Nordgren *et al.*, 1988). Bacterial activity was estimated as the rate of thymidine incorporation (TdR) into bacteria extracted from soil after homogenization and centrifugation (Bååth, 1992).

pH response of the bacterial community

To examine the extent to which the bacterial community in the field is adapted to the pH of the bulk soil (Bååth, 1996a) bacteria were separated from soils (Bååth, 1996b), incubated in two different buffers (pH 7 and pH 4), and the ratio between the rates of thymidine incorporation at the two pH levels was used as an index of the pH tolerance of the bacterial community. A high ratio was taken to indicate a community well adapted to high pH conditions, and vice versa. The pH of the bulk soil was determined in water (soil : water ratio, 1 : 5).

Field basal soil respiration

Field basal respiration rates were estimated *in situ* on May 31, June 25 and August 9, 2002, by removing gas samples sequentially from headspaces placed on the ground after removal of mosses and plant shoots (Högberg & Ekblad, 1996; Högberg *et al.*, 2001). Several locations within an area of 100 m^2 were sampled at 0, 60 and 90 m along the gradient (n = 6–10, except on May 31 in the DS forest type where n = 3).

Statistical analyses

Statistical comparisons between the three different forest types were not possible for totPLFA, SIR (first sampling) and ATP, since the TH forest type was represented by only one sampling point. However, statistical differences between the DS and SH forest types were tested by *t*-tests using the means of the F and H horizons, when these were measured separately, or the F + H, when they were not physically separated. Statistical analyses on microbial biomass-C and -N, microbial C : N ratio and field soil basal respiration rates were performed using SIGMASTAT® 2.0 (SPSS Science, Chicago, IL, USA). Effects of forest sites and sampling time were tested by two-way ANOVA using the mean values obtained from true replicates from each forest site or one-way ANOVA as appropriate (number of replicates is given). If a significant effect ($P < 0.05$) was found, Tukey's post hoc test was performed to test for significant differences among forest sites and sampling times. Means are reported ± standard deviation (SD), unless otherwise stated. Concentrations of the individual PLFAs (expressed as log mol percentage) were subjected to principal component analysis (PCA). The PLFA br18 : 0 was virtually absent in the DS to almost 1 mol% in the TH forest type and had an over-riding effect on the PCA. It was therefore excluded from subsequent PCAs. We generally focus on the differences between the DS and SH forest types, because these soils are never flooded. Moreover, although it is likely that the TH forest type is more productive than the SH type, the remaining stand is too uneven to establish such a difference. The nearly three-fold difference in forest productivity discussed in this paper is valid for the comparison between the DS and SH types.

Results

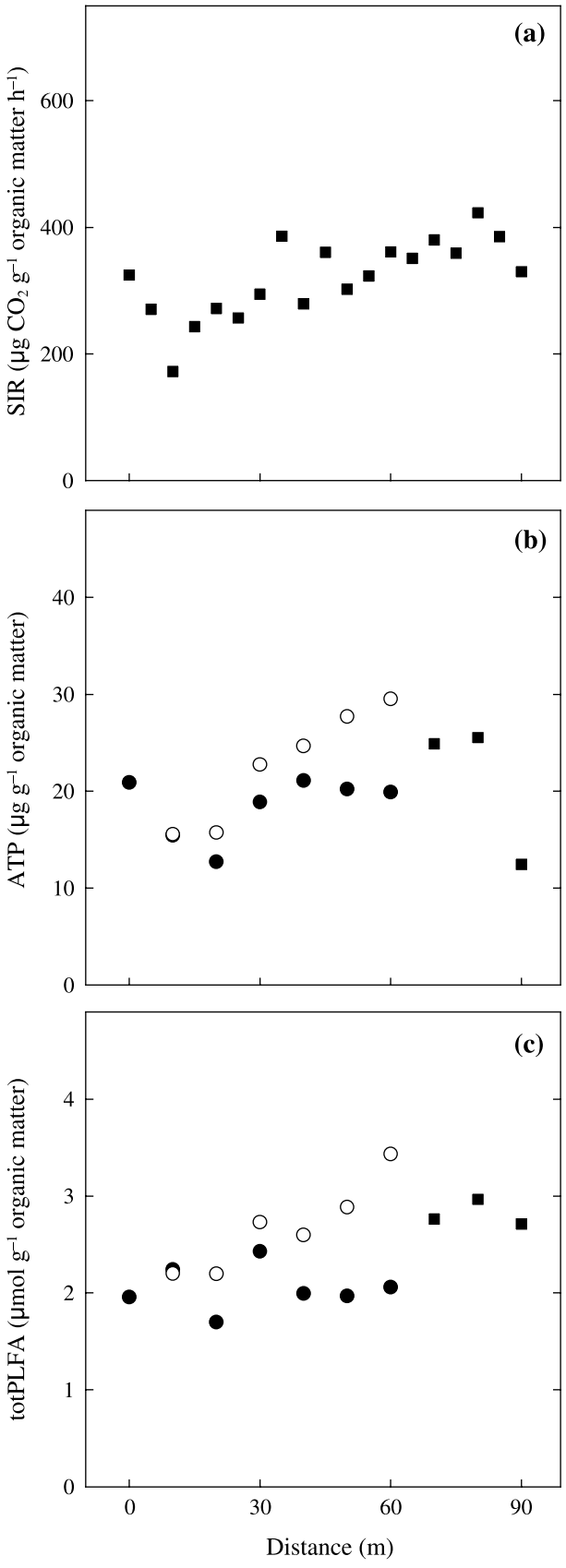
Microbial biomass

There were strong correlations between estimates of microbial biomass-C based on the totPFLA, SIR and ATP methods (Table 1, correlation coefficients (n = 16) between 0.67 and

Table 1 Selected soil microbiological properties across the 90-m-long Betsele gradient

	DS	SH	TH
Activity			
Basal respiration[a] (μg CO_2 h^{-1})	84.5 (16.2)[a]	115.9 (11.6)[a]	59.4
Basal respiration[b] (μg CO_2 h^{-1})	87.7 (4.9)[a]	112.3 (3.1)[b]	95.8 (4.2)[ab]
bactTdR (dpm ml^{-1} h^{-1} × 10^3)	90.8 (10.9)[a]	203.8 (31.1)[b]	137
Microbial biomass-C (mg)			
ATP	5.8 (0.7)[a]	7.7 (0.1)[b]	3.9
FE[c]	6.6 (1.8)[a]	3.9 (0.5)[a]	5.3 (1.1)[a]
FE[d]	10.9 (2.2)[a]	10.2 (3.5)[a]	11.0 (3.5)[a]
SIR[a]	5.9 (0.5)[a]	7.7 (0.6)[a]	6.3
SIR[b]	5.6 (0.4)[a]	7.2 (0.3)[b]	7.2 (0.6)[b]
totPLFA	6.7 (0.5)[a]	8.0 (0.3)[b]	8.0
Microbial biomass-N (mg)			
FE[d]	0.9 (0.1)[a]	1.4 (0.3)[b]	2.3 (0.1)[c]
Community structure			
bactAODC (× 10^7 cells)	8.62 (0.91)[a]	9.55 (0.07)[a]	9.61
bactPLFA (μmol)	0.69 (0.06)[a]	1.02 (0.07)[b]	1.16
C_{micr} : N_{micr}	11.7 (2.0)[a]	6.9 (1.6)[b]	4.8 (1.3)[b]
G^- : G^+ (PLFA)	0.76 (0.03)[a]	0.85 (0.05)[a]	0.77
Fungi : bacteria (PLFA)	0.44 (0.09)[a]	0.18 (0.02)[b]	0.02

Microbial-C and -N were obtained after conversion of raw data. The values are expressed per g organic matter content and represent averages (SE) for the difference forest types: DS, dwarf-shrub (0–40 m); SH, short-herb (50–80 m); TH, tall-herb (90 m); forest productivity increases three-fold between the DS and SH forest types. bactTdR, rate of thymidine incorporation into bacteria; FE, fumigation extraction; SIR, substrate induced respiration; totPLFA, total amount of phospholipid fatty acids; bactAODC, acridine orange direct counts of bacteria; bactPFLA, phospholipid fatty acids indicative of bacteria; C_{micr}, microbial-C; N_{micr}, microbial-N. Mean values not significantly different (within rows) at the P value < 0.05 are followed by the same letter. [a,b]June and July 1994, respectively. [c]June 1997. [d]Seasonal means.



0.74 ($P < 0.01$)). There was also a good agreement between absolute values based on these methods. SIR, ATP and totPLFA over the gradient increased from the DS to the SH forest type (Fig. 2), with the SH forest type having 20–30% higher microbial biomass. In the TH forest type, the microbial biomass was similar or lower than in the SH forest type (Fig. 2, Table 1). Microbial biomass-C per g^{-1} o.m. as determined by the FE method did, however, not change along the gradient. Microbial biomass expressed per area (per ha) was more variable but not significantly so ($P = 0.06$) (Fig. 3a,d). There was significantly higher microbial biomass-C (FE) values in August ($P < 0.01$ per g o.m., $P < 0.05$ per area) than in June. Microbial biomass-N increased ($P < 0.001$) from the DS to the SH forest type by 49% and from SH to the TH forest type by 64% when expressed as g^{-1} o.m. and by 30% and 230%, respectively, when expressed as microbial biomass-N per area ($P < 0.001$). Microbial biomass-N was highest in August ($P < 0.01$) (Fig. 3b).

Mean microbial biomass according to the totPFLA, SIR, ATP and FE methods was 6.7, 5.9, 5.7 and 5.3 mg biomass-C g^{-1} o.m., respectively, in June. If the C content of soil organic matter is assumed to be 45%, the microbial biomass-C accounted for 1.3–1.5% of total organic C in the mor-layer soil across this gradient. The N contents of soil organic matter in the DS, SH and TH forest types were 1.2, 2.0 and 3.1%, respectively (Table 1). Consequently the fractions of N immobilised in the microbial biomass during the vegetation period were 7.6, 6.9 and 7.3% of total soil N, respectively.

Community structure

When subjecting the PLFA data to a principal component analysis, samples from the DS forest type were found to the left along the first principal component (PC1), accounting for 41% of the variation, while samples from the TH forest type were found to the right (Fig. 4a). This is more clearly seen when the scores along PC1 are plotted against the distance along the gradient (Fig. 4c). Low scores were found for the samples from the DS forest type, while high scores, especially from the H horizon, were found for the SH and TH forest types. The second principal component (accounting for 27% of the variation) mainly differentiated between samples from the F and H horizons, when these could be separated (Fig. 4a).

The PLFAs 17 : 1ω8, 18 : 1ω9, 18 : 2ω6,9 and 20 : 4 were relatively more common in the samples from the DS forest type (Fig. 4b), while the other PLFAs, for example several iso- and anteiso-branched PLFA, as well as 10Me-branched and several mono unsaturated ones (16 : 1ω5 and 18 : 1ω7) were

Fig. 2 Microbiological properties in the F horizon (open circle), H horizon (closed circle) and F + H horizon (closed square) of the mor-layer along the Betsele forest productivity gradient (a), SIR (substrate induced respiration) (b), ATP content (c), totPLFAs (total phospholipid fatty acids).

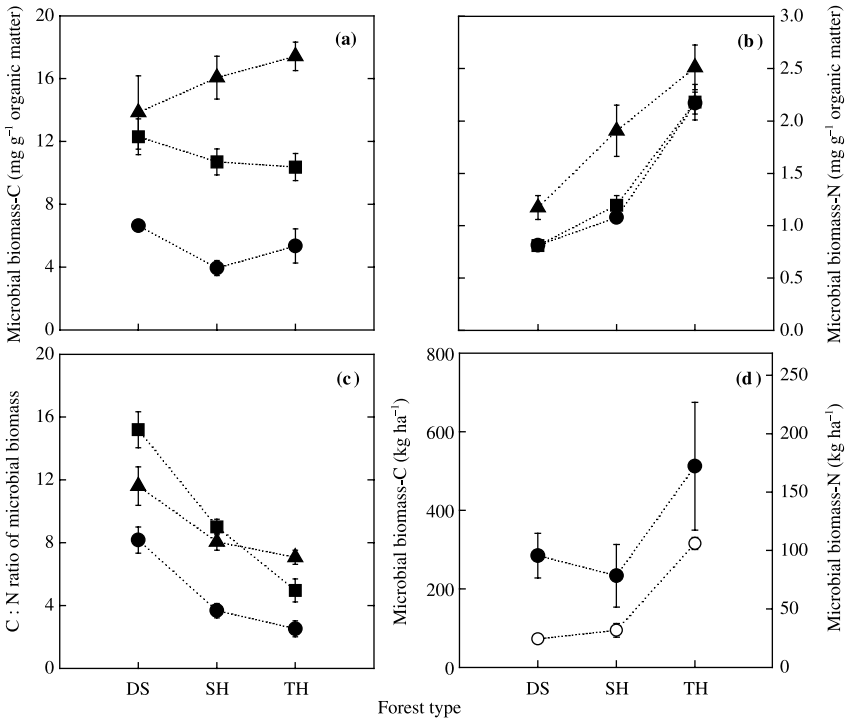


Fig. 3 Microbial biomass in the mor-layer, estimated by the FE (fumigation extraction) technique along the Betsele forest productivity gradient, June 17 (circle); August 4 (triangle); September 27 (square). (a), microbial biomass-C g^{-1} organic matter. (b), microbial biomass-N g^{-1} organic matter. (c), microbial C : N ratio. (d), microbial biomass-C (closed circle) and -N (open circle) kg ha^{-1}. Data are means ± SE.

more common in the SH and TH forest types. The fungal indicator (18 : 2ω6,9) decreased from around 15% of the total amount of PLFAs in the DS to less than 1% in the TH forest type (Fig. 5a). Another eucaryotic indicator, 20 : 4, also decreased along the gradient (Fig. 5b). Several PLFAs indicative of bacteria increased in relative amounts along the gradient, for example 18 : 1ω7, common in G^- bacteria (Fig. 5c), and 10Me18 : 0, indicative of actinobacteria (Fig. 5d). This increase was most marked with the PLFA br18 : 0 (indicative of G^+ bacteria), which was not found in several samples from the DS forest type, but increased to almost 1% of the total PLFAs in the TH forest type (Fig. 5f). The PLFA 16 : 1ω5, which has been suggested as an indicator of arbuscular mycorrhiza in soil (Olsson *et al.*, 1995; Olsson, 1999), accounted for *c.* 2% of total PLFAs in the DS forest type, and doubled in relative abundance in the TH forest type (Fig. 5e).

The sum of bacterial PLFAs increased along the gradient, being lowest in the DS and highest in the TH forest type (Table 1). Significantly more bacterial biomass g^{-1} o.m. (48%) was found in the SH compared to the DS forest types using this method. This increase in bacterial biomass was, however, not reflected in the total bacterial counts (AODC), which only increased with 10% in the SH compared to the DS forest type (Table 1). Within the microbial community, there were no changes in the ratio G^- : G^+ bacteria over the gradient (Table 1). Using the index of fungal to bacterial PLFAs suggested by Frostegård & Bååth (1996), a marked decline in fungal abundance could be seen over the gradient. In the DS forest type this index was 0.44. It decreased significantly to 0.18 in the SH forest type and became extremely low (0.02) in the TH forest type (Table 1).

In the DS forest type, the bacterial community was adapted to low pH conditions, as indicated by the low value of the pH response index (pH 7 : pH 4). This index increased along the gradient. There was a strong correlation ($r = 0.88$, $P < 0.001$) between soil pH and the bacterial community response index (Fig. 6).

The mean C : N ratio of the microbial biomass varied ($P < 0.05$) along the gradient with values of 11.7, 6.9 and 4.8 for DS, SH and TH forest types, respectively, indicating a lower abundance of fungi in the SH and TH forest types. The mean C : N ratio of the microbial biomass was highest in the autumn ($P < 0.05$) (Fig. 3c).

Microbial activity

Microbial activity determined as CO_2 released g^{-1} o.m., that is basal respiration, was significantly higher in soil from the SH compared with the DS forest type (Fig. 7a, Table 1), with increases between 28 and 37% depending on the sampling occasion. In soil samples from the TH forest type, the microbial activity was generally lower than in the SH forest type. Bacterial activity (according to the TdR incorporation) was variable (Fig. 7b), but approximately doubled in the SH compared with the DS forest type.

Root biomass

The total fine-root biomass was significantly higher in the DS compared with the SH forest type ($P < 0.001$). There was no

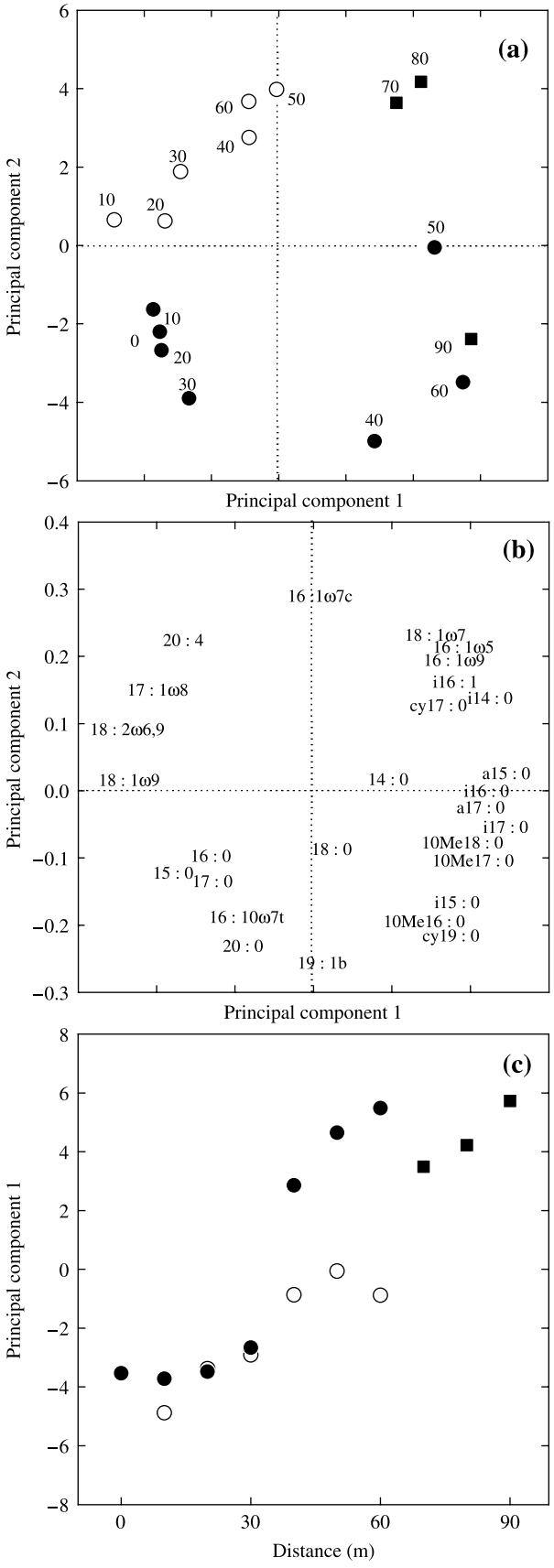


Table 2 Comparison of selected soil microbiological properties in June 1994 in F and H horizons across the Betsele gradient

	F-horizon	H-horizon	F : H ratio
Activity			
Basal respiration (μg CO_2 h^{-1})	125[a]	66.7[b]	1.87
bactTdR (dpm ml^{-1} h^{-1} × 10^3)	151[a]	82.3[b]	1.83
Microbial biomass-C (mg)			
ATP	7.1[a]	5.6[b]	1.26
SIR	7.5[a]	5.6[b]	1.34
totPLFA	7.9[a]	6.1[b]	1.30
Community structure			
bactAODC (× 10^7 cells)	10.7[a]	7.24[b]	1.47
bactPLFA (μmol)	0.82[a]	0.67[b]	1.22
G^- : G^+ (PLFA)	0.85[a]	0.69[b]	1.24
Fungi : bacteria (PLFA)	0.48[a]	0.27[b]	1.77

Microbial-C and -N were obtained after conversion of raw data. Samples were from the dwarf-shrub (DS) forest type and short-herb (SH) forest type, where the F and H horizons could be sampled separately $n = 6$, except for basal respiration and SIR, where $n = 7$. Data are expressed per g organic matter content. F : H indicates the ratio between the microbiological variables in the two horizons. bactTdR, rate of thymidine incorporation into bacteria; SIR, substrate induced respiration; totPLFA, total amount of phospholipid fatty acids; bactAODC, acridine orange direct counts of bacteria; bactPFLA, phospholipids indicative of bacteria. Values not significantly different (within rows) are followed by the same letter (paired *t*-test, $P < 0.05$).

difference in the coniferous tree fine-root biomass between the DS and SH forest types, but the tree fine-root biomass was lower in the TH forest type than in the SH type ($P < 0.01$, Fig. 8a).

Basal respiration in the field

There were no significant differences in field respiration rates between forest types ($P = 0.089$). Rates showed a seasonal trend with significantly higher rates throughout the gradient late in the summer ($P < 0.01$) (Fig. 8b).

Comparison between the F and H horizons

The microbiological properties tested showed higher values in the F than in the H horizon (Table 2). The largest differences (up to 1.8 times) were found amongst the activity indicators (respiration, bacterial TdR incorporation), while biomass measurements (both total biomass and bacteria

Fig. 4 Principal component analysis of the microbial community composition in the F horizon (open circles), H horizon (closed circles) and F + H horizon (closed squares) of the mor-layer across the gradient as indicated by phospholipid fatty acids (PLFAs). The first component is related to distance, while the second component is related to soil horizon. (a), sample ordination. (b), PLFA ordination. (c), sample scores on the first component plotted against the distance along the gradient.

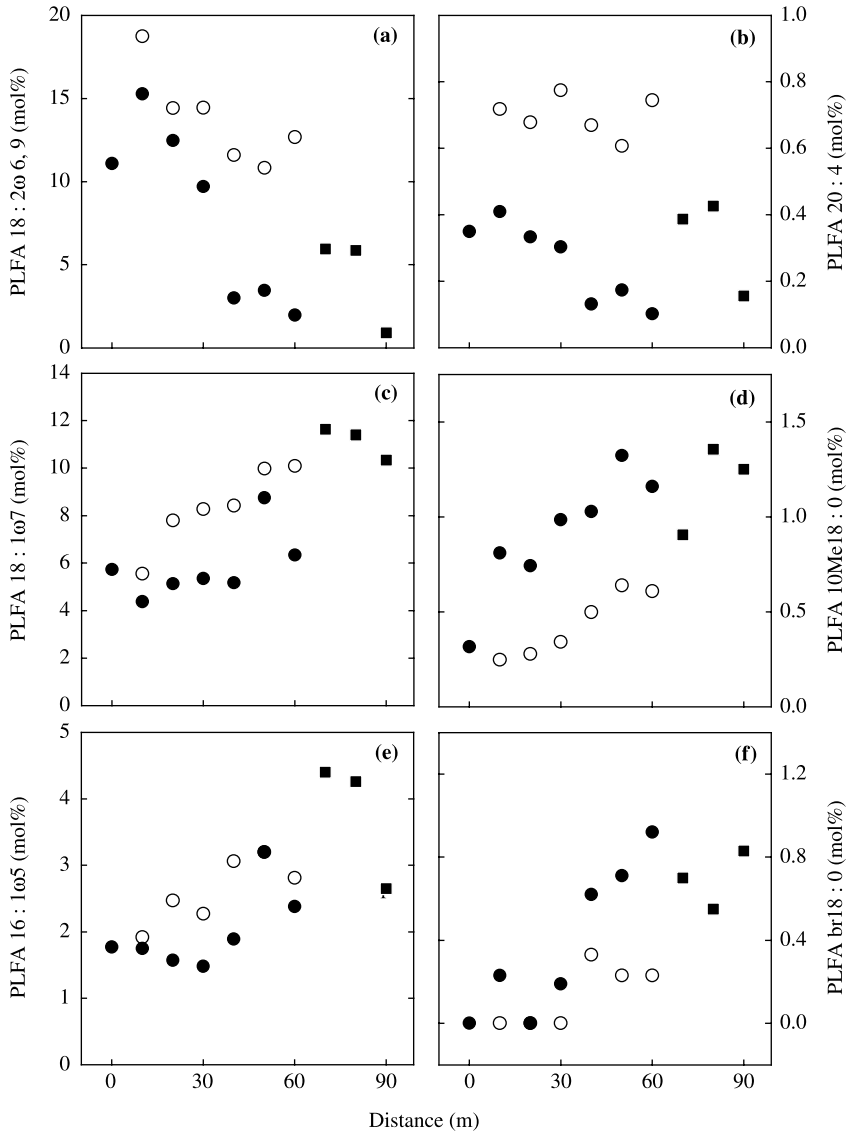


Fig. 5 Mol percentage of individual phospholipid fatty acids (PLFAs) in the F horizon (open circles), H horizon (closed circles) and F + H horizon (closed squares) of the mor-layer along the Betsele forest productivity gradient. (a), the fungal indicator 18 : 2ω6,9. (b), eucaryotic indicator 20 : 4. (c), gram negative (G^-) bacterial indicator 18 : 1ω7. (d), actinobacterial indicator 10Me18 : 0. (e), arbuscular mycorrhiza indicator 16 : 1ω5. (f), gram positive (G^+) bacterial indicator br18 : 0.

specific biomass) were around 1.3 times higher in the F than in the H horizon. There were also large differences in the microbial community composition (Fig. 4a,b), where the F horizon had relatively higher amounts of the PLFAs 16 : 1ω7c, 18 : 1ω7 (Fig. 5c), 20 : 4 (Fig. 5b), while the H horizon, especially, had relatively higher amounts of the PLFAs 19 : 1b, 20 : 0, cy19 : 0 and 10Me16 : 0, 10Me17 : 0 and 10Me18 : 0 (Fig. 5d). There was a lower ratio fungi: bacteria in the H horizon compared with the F horizon (Table 2).

Discussion

The microbial biomass-C, -N and microbial C : N ratios in soil organic matter were in the range found in other coniferous forests. The mean microbial-C across the gradient at Betsele, estimated by the four methods, was 1.4 ± 0.1% of total organic C. This was close to the 1.7% found in a coniferous boreal forest of similar age (Bauhus *et al.*, 1998), the 1.2% in Finnish Pinus forests (Martikainen & Palojärvi, 1990), the 1.6% in Finnish Picea stands (Smolander & Mälkönen, 1994) but lower than the 2.2% based on FE (64 studies), SIR (nine studies) and microcalorimetry (one study), previously found for boreal forests (Bauhus & Khanna, 1999). The mean contribution of microbial-N to total N was 7.3%; almost the same concentration (7.5%) was found by Bauhus *et al.* (1998) in the forest floor of coniferous boreal forests. The mean microbial C : N ratio over the Betsele gradient was *c.* 8 compared with 9 in the study by Bauhus *et al.* (1998).

In the following, we will concentrate on differences in microbial biomass, community structure and activity between the DS and SH forest types, which show a three-fold difference in plant productivity. The TH forest type has significantly different forest and soil conditions (more open,

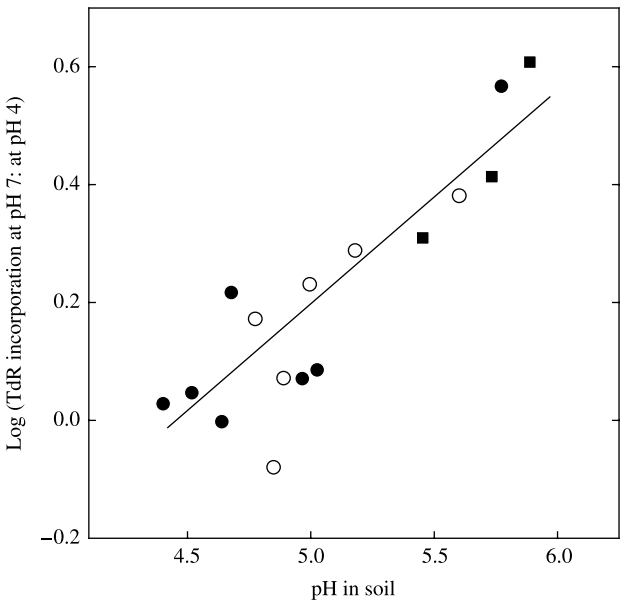


Fig. 6 Bacterial tolerance along the natural soil pH gradient estimated as the logarithm of the ratio of the thymidine incorporation rate of the bacterial community at pH 7 divided by the rate found at pH 4, that is [log (pH 7: pH 4)]. F horizon (open circles); H horizon (closed circles); and F + H horizon (closed squares).

periodically water-logged) compared to the rest of the gradient. Periodic anaerobic conditions and/or very high concentrations of N (Berg & McClaugherty, 2003) may, for example, explain the greater soil organic matter accumulation in the TH forest.

The most dramatic change observed was the decrease in the fungal PLFA 18 : 2ω6,9, from around 15 mol% in the DS forest to around 1% in the TH forest (Fig. 5a). One reason for this decrease could be the pH increase across the gradient (Fig. 1), because fungi are favoured compared with bacteria at low pH (Alexander, 1977; Hartel, 1999). In beech-oak forest, (Blagodatskaya & Anderson, 1998) found an increase in the ratio fungi : bacteria (measured with the selective inhibition technique) with decreased pH. However, elsewhere using the PLFA or ergosterol methods to estimate fungal abundance, only small effects were found after considerable pH changes which were induced by liming, ash-treatment or alkaline pollution of coniferous forest soil (Fritze & Bååth, 1993; Frostegård *et al.*, 1993; Bååth *et al.*, 1995). In a recent study Bååth & Anderson (2003) found that the effect of pH on the ratio fungi:bacteria depended on the method used (PLFA or selective inhibition). Thus, even in a natural pH gradient ranging over four units, there was no correlation between pH of the bulk soil and the fungal indicator PLFA 18 : 2ω6,9. Hence, the decreasing fungal biomass observed in direction of the groundwater discharge area at Betsele may not be a direct effect of soil pH per se.

There are, however, several other estimates of microbial community structure and activity that vary with soil pH in

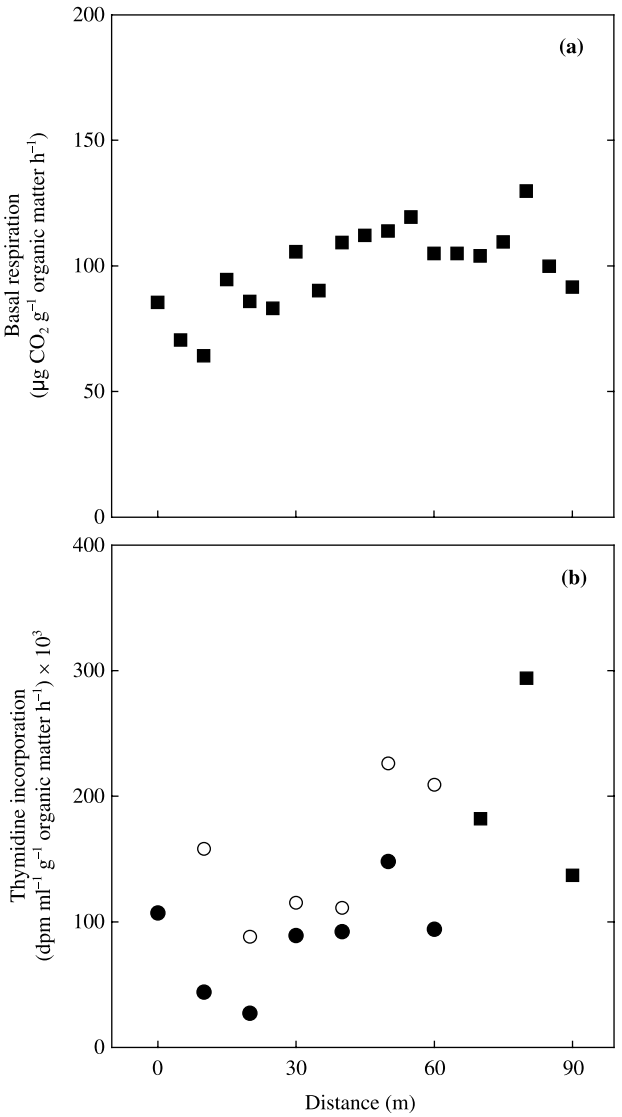


Fig. 7 Microbial activity in sieved soil from the F horizon (open circles), H horizon (closed circles) and F + H horizon (closed squares) of the mor-layer along the Betsele forest productivity gradient. (a), basal soil respiration measured in the laboratory. (b), bacterial activity estimated as thymidine (TdR) incorporation rate.

our study, and most likely are direct effects of pH. The most self-evident is the pH tolerance of the bacterial community (Fig. 6). This has earlier been shown over a range of different soils (Bååth, 1996a). Changes in the bacterial community structure, as revealed by PLFA, also appeared pH-related, for example variations in the PLFA 10Me18 : 0 (Fig. 5d), indicative of actinobacteria, which are known to prefer higher pH. The difference in PCA loadings for PLFAs along the second principal component (Fig. 4b), which differentiated between F and H horizons, could also partly be a pH effect, since the PLFAs affected at Betsele were largely those also found to be related to pH in deciduous forests (Bååth & Anderson, 2003).

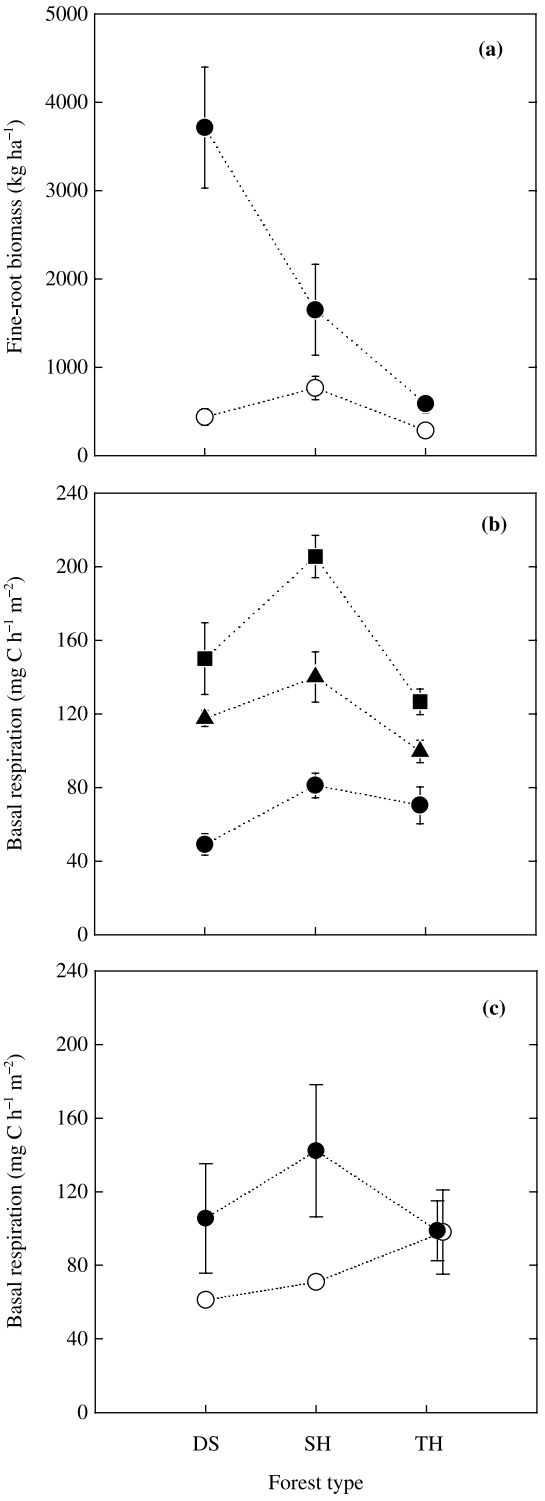


Fig. 8 Fine-root biomass and soil basal respiration measured in the field and in the laboratory in the different forest types along the Betsele gradient. (a), total fine-root biomass (filled circles), Pinus and Picea fine-roots (open circles). (b), basal respiration rate estimated in the field in 2002: May 31 (closed circles), June 25 (closed triangles) and August 9 (closed squares). (c), mean field basal respiration rates (closed circles) and mean basal respiration rate measured in the laboratory (open circles). Data are means ± SE.

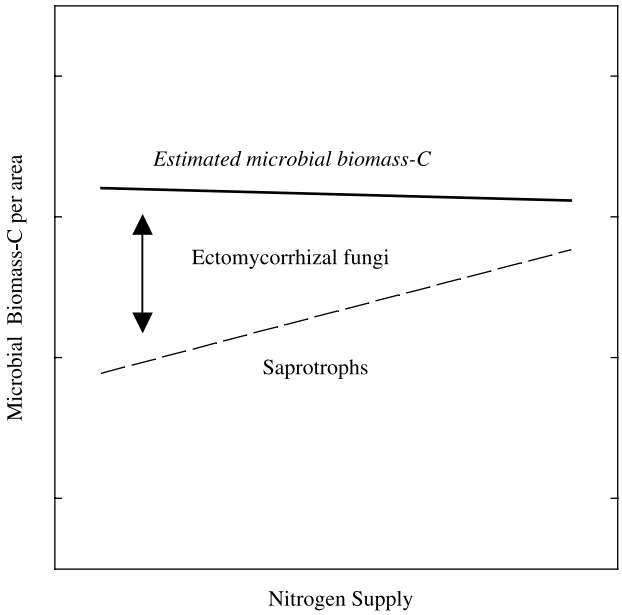


Fig. 9 A model of changes in the size of the microbial biomass and shifts between functional groups when soil N supply increases from the DS to the SH forest types (the model exludes the TH forest type since it represents extraordinary conditions, see text for further discussion). The model represents the range in microbial biomass-C data in June, based on the means of the four methods ATP, SIR, totPLFA and FE (solid line). The contribution by ectomycorrhizal fungi in the DS forest type is taken from a study of a similar forest (Högberg & Högberg, 2002); the decrease in the contribution of these fungi relates to the three-fold decline, from approximately 15–5 mol%, of the PLFA 18 : 2ω6,9 between the DS and SH forest types (broken line). The arrow illustrates temporal variation in the size of the soil microbial biomass and the suggested sensibility of the mycorrhizal fungal component to disruption of its major C supply caused by physical disturbance, for example as a consequence of soil sampling.

Increased microbial activity (e.g. basal respiration and bacterial TdR incorporation rate, Table 1) in the SH compared with the DS forest types could also be an effect of higher soil pH.

However, other factors in addition to soil pH also vary substantially along the gradient, for example N supply, plant species composition and productivity. Since microorganisms generally are thought to be C-limited, the proximal controls on their C-supply must be considered in this context. Soil pH is likely to influence the C-supply to soil microorganisms indirectly through effects on soil N turnover, plant productivity and plant community composition, and thereby the quantity and quality of plant litter (Högberg, 2001). Direct effects of pH on the soil C-supply to the microorganisms are possible, but have not been clarified. The profound effect of N-supply on plant productivity (and hence ultimately on the total C-supply to soil saprotrophs) in boreal forest is, on the other hand, well established. The effect of N-supply, which here is correlated with soil pH, apparently also is a determinant of the plant community structure, and hence of the type of mycorrhiza formed. We have therefore chosen to focus on

these obvious controls of the C-supply to soil microorganisms, rather than a less clear link between soil pH and the C-supply to soil microorganisms.

We expected to observe an increase in microbial biomass both per g organic matter and per unit area, in response to increasing plant productivity and substrate quality. Microbial biomass estimates based on SIR, ATP and totPLFA indicated differences in microbial C along the gradient with higher values in the SH forest type than in the DS type both on an area basis and when expressed as g^{-1} organic matter. By contrast to these three estimates, the FE method, which was applied to soil shortly after sampling, showed no significant variation in microbial biomass C along the gradient. The reason for this discrepancy may be that mycorrhizal fungi, which are disconnected from their hosts during sampling, rapidly lose activity and biomass, while saprotrophs will largely remain attached to their C sources. The FE method was applied more quickly after soil sampling than the other methods. Consequently, the data obtained by the FE method may better reflect the microbial biomass under natural undisturbed conditions than the other methods used. The increase in microbial biomass as measured by the totPLFA, ATP and SIR methods, was small, that is around 20–40%, in relation to the much larger increase in plant productivity along the gradient. We expected that there should be an increase in microbial biomass in proportion to the three-fold increase in plant productivity from the DS- to the SH-forest type, but had to reject this initial hypothesis (Fig. 9).

The SIR, ATP and totPLFA methods gave higher biomass values g^{-1} organic matter, indicating a higher substrate quality towards the more productive end of the gradient. Such an increase should be expected when, as observed here, the soil C : N ratio declines from 39 to 17. This trend was not found in the FE data. Again, this discrepancy may reflect the different extent to which fungal mycorrhizal biomass-C was measured by the different approaches.

The resolution to these apparent contradictions is evidently in the differences in the ratio of fungi:bacteria. The PLFA 18 : 2ω6,9, thought to indicate ectomycorrhizal, ericoid mycorrhizal and other fungi, decreased drastically from the DS to the TH forest type, while bacteria increased. Mycorrhizal fungi receive photosynthate C from their hosts, that is a high quality substrate, under conditions of low litter substrate quality in the DS forest type. Consequently, the combined substrate quality in the DS forest environment may equal that in the TH forest type. The C supply to ectomycorrhizal fungi, especially to the extraradical mycelium, is thought to be reduced under conditions of high nutrient supply (Nylund, 1988; Wallander, 1995). This could be the case at the more productive end of the gradient. There, the supply of readily available photosynthates to mycorrhizal fungi should be less and then activity declines, but this is balanced by a larger supply of high quality litter to saprotrophs, which leads to an increase in their activity. These arguments were supported by first the absence of any major trend in soil (root and microbial) *in situ* respiration rates across the gradient and second the marked decline in the biomass of total fine-roots across the gradient. This argument is also supported by the decline in relative allocation of photosynthates to roots following fertilization in a N-limited Pinus forest (Cannell, 1989). This should result in a decline in ectomycorrhizal fungal activity as shown in the laboratory by Arnebrant (1994), and by Kårén & Nylund (1997) and Nilsson & Wallander (2003) in other forest fertilization experiments. A recalculation of soil basal respiration rates from our laboratory incubations (per g o.m.) gave surprisingly equal rates to those found in the field (per area) in June, when the microbial biomass seemed to be lowest (Figs 3a, 7a and 8b,c). Field rates in August and September in the DS and SH forest types were clearly higher than rates measured in the field or in the laboratory in June, while no difference was observed for the TH forest type (Fig. 8c). This indicates that the TH soil is less sensitive to sampling and incubation than the other soils, which might be expected when the biomass of mycorrhizal mycelium is low.

Surprisingly, the larger above-ground plant biomass towards the productive end of the gradient at Betsele does not support larger soil microbial activity (via root allocation and above-ground litter fall) even if the relative allocation of photosynthates to roots is reduced. However, the stand girdling experiment (Högberg *et al.*, 2001) in strongly N-limited boreal forest suggests that the respiration of mycorrhizal roots is equal to or even exceeds saprotrophic microbial respiration. Hence, the reduction in the activity of mycorrhizal fungi towards the high productive end of the gradient could be significant.

In a microbial community with a significant mycorrhizal fungal component, which is dependent on the flux of photosynthates from the plants, we expect a seasonality, depending on the C allocation to the roots with a maximum in late summer (Hansen *et al.*, 1997). This seasonality of the flux of C may thus be a reason why the microbial biomass C and soil respiration rates in the field were highest in August. As found by (Söderström, 1979; Bååth & Söderström, 1982) fluorescein diacetate (FDA) active fungi were most abundant in early spring and autumn. Wallander *et al.* (2001) measured the highest ingrowth rates by ectomycorrhizal extramatrical mycelium into mesh-bags in the autumn.

By contrast to the above, microbial biomass-N ha^{-1} increased several-fold along the gradient. This was expected along a three-fold gradient in plant productivity and also supports the generality of the finding of a highly significant positive correlation between microbial biomass-N and total N in the forest floor among tropical, temperate and boreal forests (Bauhus & Khanna, 1999). Microbial biomass-N also showed seasonal differences in our study, but they were less pronounced than those for C. We thus suggest that the C supply to microbes is more variable than the N supply, especially since the C supply to mycorrhizal fungi is highly variable.

Read (1991) described regular variations in type of mycorrhiza formed across large latitudinal and altitudinal gradients as follows. Under cold and wet conditions soils become leached and acid and N mineralization is slow; there ericoid mycorrhiza and ectomycorrhiza are the dominant types. Under warm and drier conditions decomposition is more complete, soils are less leached and less acid; there arbuscular mycorrhiza is often dominant. At Betsele we observed a doubling of the signature PLFA for arbuscular mycorrhiza in direction of increasing pH and abundance of short and tall herbs. At the same time the signature PLFA encompassing ectomycorrhiza and ericoid mycorrhiza decreased several-fold. Hence, the associations between certain soil conditions, plants and certain mycorrhizal types proposed by Read can also be found within very short distances.

Conclusions

Our observations heighten the need to recognize differences in factors determining the activities of different functional groups and the roles that they play in soil microbiology and forest ecology. We hypothesize that opposing nutrient limitations govern C-supplies to saprotrophic microorganisms and mycorrhizal fungi. The former increase when their supply of C from plants is enhanced in response to greater N-supply; on the contrary, increased N-supply seems to cause a reduction of the C-supply to plant roots and their mycorrhizal symbionts. Our hypothesis could be tested by experiments (e.g. tree girdling) clarifying the effect of variations in soil N availability on the fractional contribution of respiration by ectomycorrhizal roots to total soil respiration. Our study also points out the need to quantify the loss of the biomass and activity of mycorrhizal fungi after soil sampling.

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