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Composition of the microbial communities in the mineral soil under different types of natural forest

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Abstract

Phospholipid fatty acid (PLFA) patterns were used to describe the composition of the soil microbial communities under 12 natural forest stands including oak and beech, spruce–fir–beech, floodplain and pine forests. In addition to the quantification of total PLFAs, soil microbial biomass was measured by substrate-induced respiration and chloroform fumigation–extraction. The forest stands possess natural vegetation, representing an expression of the natural site factors, and we hypothesised that each forest type would support a specific soil microbial community. Principal component analysis (PCA) of PLFA patterns revealed that the microbial communities were compositionally distinct in the floodplain and pine forests, comprising azonal forest types, and were more similar in the oak, beech and spruce–fir–beech forests, which represent the zonal vegetation types of the region. In the nutrient-rich floodplain forests, the fatty acids 16:1ω5, 17:0cy, a15:0 and a17:0 were the most prevalent and soil pH seemed to be responsible for the discrimination of the soil microbial communities against those of the zonal forest types. The pine forest soils were set apart from the other forest soils by a higher abundance of PLFA 18:2ω6,9, which is typical of fungi and may also indicate ectomycorrhizal fungi associated with pine trees, and high amounts of PLFA 10Me18:0, which is common in actinomycetes. These findings suggest that the occurrence of azonal forest types at sites with specific soil conditions is accompanied by the development of specific soil microbial communities. The study provides information on the microbial communities in undisturbed forest soils which may facilitate interpretation of data derived from managed or even damaged or degraded forests.

Keywords: Chloroform fumigation–extraction, Microbial biomass; Microbial community composition; Natural forest soil; PLFA; SIR

1. Introduction

A wide range of bedrock materials and climate conditions are found in Austria between the eastern end of the Alps and the Hungarian lowlands, where there are various forest types of biogeographical relevance. At undisturbed sites with natural vegetation, forest reserves have been established in order to conserve natural plant communities and to provide scientific study sites. Since management is excluded from these natural forests, ecosystem development and functioning are widely unaffected by anthropogenic influences.

Soil microorganisms represent essential components of the biotic system in natural forests where they are key players in nutrient turnover (Zechmeister-Boltenstern et al., 2000; Hackl et al., 2004). Even so, how microbial communities interact transformations has rarely been studied. Many microbial processes are interactive, with individual species relying on the presence and activities of other species. As different functional groups of microorganisms respond differently to prevailing environmental conditions, forest stand characteristics (i.e. soil and vegetation properties) influence the composition of the soil microbial community in a specific way.

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To study the composition of the microbial communities in soils, community-level microbial analyses have been applied. Thereby, the use of traditional counting and naming techniques was excluded because usually only 6.5% of the bacteria present in a soil sample can be characterised by selective culturing (Bakken, 1995). A culture-independent approach to studying the composition of soil microbial communities is offered by the analysis of the phospholipid fatty acid (PLFA) compounds of the microbial membranes (Federle, 1986; Frostegård et al., 1991; Tunlid and White, 1992; Zelles et al., 1992). Subsets of the microbial community differ in their PLFA patterns, which makes it possible to plot signatures of the microbial communities in their habitats.

In forest soils, PLFA analysis has been applied to elucidate changes in the microbial community composition along a fertility gradient (Pennanen et al., 1999) and in alliance with changes in vegetation along a successional chronosequence (Ohtonen et al., 1999). Forest soil PLFA patterns have been found to change in response to liming, application of wood-ash and burning (Frostegård et al., 1993; Bååth et al., 1995). Effects on PLFA profiles have also been reported through disturbance caused by forest management practices (Ponder and Tadros, 2002).

The natural forests considered in our study represent the clearest expression of the natural site factors among various landuse types found in this region. We hypothesised that natural forest sites supporting specific plant communities would also select for microorganisms with specific environmental requirements. Different types of natural forest would thus be associated with characteristic soil microbial communities. Information on the microbial community composition in undisturbed forest soils could then be used to facilitate interpretation of microbial community data derived from measurements in managed or even damaged or degraded forests.

Our objective was to assess the qualitative and quantitative characteristics of the soil microbial communities under 12 representative natural forest stands by PLFA profiling and measurement of microbial biomass. Since the flora of the stands had not been changed artificially, the soil properties were presumed to be well differentiated according to vegetation and site conditions. Thus, we analysed the composition of the microbial communities in relation to a complex set of variables, including soil and vegetation characteristics as well as climatic conditions, which define the different forest types.

2. Material and methods

2.1. Sampling sites

The 12 forest stands are all situated within the eastern part of Austria and feature oak–hornbeam, woodruff–beech, acidophilous beech, spruce–fir–beech, floodplain and Austrian pine forests, which are also common throughout Europe. The oak and the beech forests comprise the zonal vegetation types found in the region and therefore reflect the regional climate (Ellenberg, 1996). The zonal vegetation types are distributed along a thermal gradient: oak–hornbeam forests are favoured by warm and dry conditions and are succeeded by beech forests and further by spruce–fir–beech forests as the climate gets colder and wetter. These forest types have developed on soils which are not waterlogged or temporarily flooded or show any extreme properties such as strong nutrient limitation. In addition, two azonal vegetation types typical of dry and wet sites have been included, namely pine and floodplain forests. Azonal vegetation communities may occur in several different climatic regions because they are determined by the same extreme soil factors (Ellenberg, 1996).

Since the selected forest types are widely distributed throughout Austria, non-uniformity within the same vegetation type may exist in soil type, small-scale climatic conditions, altitude, etc. Each forest type was studied at two sites with similar tree species composition, which hence may still differ in some environmental variables. As for the beech forests, which have the most extensive distribution, we distinguished between woodruff–beech forests situated on calcaric bedrock and ‘acidophilous’ beech forests situated on acidic bedrock. An overview of the site characteristics and the geographical locations is given in Table 1 and vegetation characteristics are presented in Table 2. In all stands studied the vegetation composition is considered to be natural in terms of not being changed by human activities. Most of the stands have been designated as natural forest reserves, which means that they have been excluded from forest management. The ‘Klausen-Leopoldsdorf’ beech forest represents a EU Level 2 study site and like the two pine forest stands is managed very extensively. The sites at Rothwald and Neuwald are virgin spruce–fir–beech forests that have never been used for wood-production. Soils were classified according to FAO nomenclature (1994) and plant associations according to Mucina et al. (1993).

2.2. Sampling procedure

Soils from each stand were sampled between 16 April and 1 July (spring sampling) and again between 17 Sept. and 29 Oct. (autumn sampling). In spring, we chose the time shortly after foliation had begun while in autumn it was the onset of litter fall. To ensure that the seasonal phenology was similar for the 12 forest sites at the various sampling dates, sites situated at higher elevations were sampled later in spring and earlier in autumn relative to sites at lower elevations. At each stand, a transect of 50 m was established and, at intervals of 5 m, 10 soil samples were taken from the mineral layer (0–10 cm depth) after the litter had been removed. In autumn, the transects were shifted 1 m from the previous ones to guarantee undisturbed sampling. Thus, 120 soil samples (10 per site) were gathered at each season,

Table 1
Site characteristics of the forest stands under study

Forest type	Site[a]	Geographical location	Elevation (m a.s.l.)	Annual climate data		Soil type	Geology
		(lat. N, long. E)		Temp. (°C)	Ppt. (mm)		
Oak–hornbeam	JE	48°11′N, 16°13′E	325	8.8	643	Dystric Planosol	Laab formation
	K	47°58′N, 16°41′E	270	8.7	593	Calcaric Planosol	Micashist/Limestone
Woodruff–beech	JB	48°11′N, 16°13′E	320	8.8	643	Dystric Planosol	Laab formation
	Kl	48°07′N, 16°03′E	510	7.6	768	Dystric Cambisol	Laab formation
Acidophilous beech	D	48°24′N, 15°32′E	500	7.6	613	Dystric Cambisol	Gföhl gneiss
	S	48°32′N, 15°33′E	550	7.4	631	Dystric Cambisol	Gföhl gneiss
Spruce–fir–beech	R	47°46′N, 15°07′E	1035	5.5	1759	Chromic Cambisol	Dolomite
	N	47°46′N, 15°32′E	995	5.8	1262	Stagnic Luvisol	Sandstone
Floodplain	M	48°00′N, 16°42′E	160	9.7	582	Calcaric Fluvisol	Recent clay
	B	48°08′N, 16°33′E	160	9.7	534	Calcaric Fluvisol	Recent clay
Austrian pine	St	47°53′N, 16°02′E	640	7.0	668	Rendzic Leptosol	Dolomite
	Me	47°59′N, 16°10′E	475	8.2	554	Rendzic Leptosol	Dolomite

Climate data include mean annual temperature (Temp.) and total annual precipitation (Ppt.) from long-term averages (Hackl et al., 2004).
[a] JE, Johannser Kogel Oak; K, Kolmberg; JB, Johannser Kogel Beech; Kl, Klausen-Leopoldsdorf; D, Dürnstein; S, Saubrunn; R, Rothwald; N, Neuwald; M, Müllerboden; B, Beugenau; St, Stampfltal; Me, Merkenstein.

which were treated individually. The soil samples were taken to the laboratory in cooling boxes. They were sieved (2 mm mesh) and then stored in aliquots. For SIR measurements, aliquots of all samples were kept at 4 °C and were analysed within 4 weeks after the sampling. The remaining aliquots were stored at −20 °C for subsequent analyses. Prior to use, the soil samples were defrosted at 4 °C and, for the FE-biomass measurements, were left to equilibrate at room temperature.

2.3. Chemical analyses

Soil dry weight was determined by drying subsamples at 105 °C overnight. Soil pH was measured in deionised water by a glass electrode. Percent total soil carbon (C_t) and total soil nitrogen (N_t) were analysed after dry combustion, whereby percent organic carbon (C_{org}) was calculated by subtracting carbonate content from C_t.

2.4. Microbiological analyses

Microbial biomass N was determined by a chloroform fumigation–extraction technique and was calculated as ninhydrin-reactive N*3.1 (Hackl et al., 2000). Microbial biomass C was calculated from the maximum initial respiratory response of substrate-induced respiration (SIR) with glucose-amendment measured by an infra red gas analyser as described by Hackl et al. (2000). Low molecular weight organic compounds of the soil organic matter (sugars, amino acids) were analysed in extracts of 60% acetone according to Hackl et al. (2000). NO_3^-–N was measured colorimetrically as NO_2–N after enzymatic reduction using the test kit by Mannheim-Boehringer.

Ergosterol as a biomarker for fungi in soil was extracted by 96% ethanol without further saponification. Quantitative determination was performed by reversed phase-HPLC and UV detection according to Djajakirana et al. (1996).

Table 2
Vegetation characteristics of the forests studied (Hackl et al., 2004)

Site	Forest community	Main tree species
JE	Carpinion	*Carpinus betulus* L., *Quercus petrea* Liebl., *Quercus cerris* L., *Acer campestre* L., *Ulmus glabra* Huds.
K	Carici pilosae-Carpinetum	*Q. petrea* Liebl., *C. betulus* L., *Fagus sylvatica* L.
JB	Eu-Fagenion	*F. sylvatica* L., *C. betulus* L.
Kl	Hordelymo-Fagetum	*F. sylvatica* L.
D	Luzulo-Fagenion	*F. sylvatica* L.
S	Luzulo-Fagenion	*F. sylvatica* L.
R	Adenostylo glabrae-Fagetum	*F. sylvatica* L., *Abies alba* Mill., *Picea abies* Karsten
N	Cardamino trifoliae-Fagetum	*F. sylvatica* L., *A. alba* Mill., *P. abies* Karsten
M	Pruno-Fraxinetum	*Fraxinus excelsior* L., *Alnus glutinosa* Gaertn., *Prunus padus* L., *A. campestre* L., *Salix alba* L.
B	Fraxino-Populetum	*Populus alba* L., *Acer negundo* L., *F. excelsior* L.
St	Euphorbio saxatilis-Pinetum nigrae	*Pinus nigra* Arnold
Me	Euphorbio saxatilis-Pinetum nigrae	*P. nigra* Arnold

For abbreviations of study sites see Table 1.

Phospholipid extraction and phospholipid fatty acids (PLFAs) analysis were done as described by Frostegård et al. (1991) with some modifications. To summarise this procedure, 1 g fresh weight of soil was extracted with chloroform:methanol:citrate buffer mixture (1:2:0.8) and the lipids were separated into neutral lipids, glycolipids, and phospholipids on a silicic acid column. The phospholipids were subjected to a mild alkaline methanolysis, and the fatty acid methyl esters were detected on a Hewlett-Packard 5890 II gas chromatograph equipped with a flame ionisation detector. The column used was a HP 5 (50 m × 0.20 mm × 0.33 μm) capillary column. Helium was used as the carrier gas. The temperatures of the injector and detector were 280 and 350 °C, respectively. Samples (1 μl) were injected in the splitless mode. An initial oven temperature of 70 °C was maintained for 2 min, then raised to 160 °C at 30 °C min^{-1} and then increased to the final temperature of 280 °C at 3 °C min^{-1} for 15 min. Peak areas were quantified by adding methyl non-adecanoate fatty acid (19:0) as an internal standard. The identification of the fatty acid methyl esters was based on comparison with chromatograms of fatty acid methyl esters standard compounds (Bacterial Acid Methyl Esters Mix from SUPELCO) and on the structural analysis of their 4,4-dimethyloxazoline derivatives performed by gas chromatography mass spectrometry as described by Fay and Richli (1991) and Spitzer (1997). Mass spectrometric analyses were carried out with a Hewlett Packard 5890 II/5971A GC/MS system.

Fatty acids are designated in terms of 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 i and a indicate iso- and anteiso-branching, cy indicates cyclopropane fatty acid. Me refers to the position of methyl group from the carboxyl end of the chain.

The total amount of PLFAs was used to indicate the total microbial biomass and the sum of the PLFAs considered to be predominantly of bacterial origin (i15:0, a15:0, 15:0, i16:0, 16:1ω7, 16:1ω5, i17:0, a17:0, 17:0cy, 17:0, 18:1ω7, 19:0cy) were chosen to represent bacterial biomass (Frostegård et al., 1993). The quantity of the fatty acid 18:2ω6,9 was used as an indicator of fungal biomass, since it is suggested to be mainly of fungal origin in soil (Olsson, 1999). Based on the findings by Kroppenstedt (1985), the fatty acid 10 Me18:0 was used to indicate actinomycete biomass.

2.5. Statistical analyses

Statistica 5.1 was used for the statistical data processing. Since homogeneity of variances was not ensured, the non-parametric Kruskal-Wallis H-test and the Mann-Whitney U-test were used to test for significant differences between forest sites and sampling dates. Correlations between microbial biomass values and soil chemical variables were determined using Spearman's rank correlations. In correlations of microbial biomass with soil environmental factors, all data were calculated on a soil organic C basis to eliminate the effect of SOM.

The mole percent of the individual PLFAs from spring and autumn, respectively, was subjected to principal component analysis (PCA) to elucidate major variation patterns. In the PCA of the spring data, clockwise rotation of the first two components by 45° was performed, whereas PCA of the autumn data was done without rotation. The scores of the first two components from the PCA from both seasons were then used to compare differences between the forest stands in the PLFA patterns with differences in soil chemical properties, climate data and in biomass content g^{-1} organic C.

3. Results

3.1. Microbial biomass

Data on microbial biomass gained by the different methods gave different information with respect to seasonal variability: For some sites, differences between spring and autumn sampling were more pronounced in FE-biomass than in SIR-biomass or total PLFAs, while for other sites data on FE-biomass were more uniform in spring and autumn relative to data on SIR-biomass or total PLFAs (Fig. 1). For instance, differences in microbial biomass in the pine forest soils between spring and autumn sampling were more pronounced by SIR than when estimated as total PLFAs and were minor when measured by FE. The content of SIR-biomass in the floodplain forest soils, in contrast, showed less variation between spring and autumn sampling compared to total PLFAs and FE-biomass.

The content of microbial biomass in the soils strongly corresponded to the soil organic matter (SOM) content. Hence, the 'Rothwald' forest soil, which was highest in organic C (Tables 3 and 4), also had the highest concentration of total PLFAs, exceeding averages in the other soils more than two-fold (Table 5).

Total PLFAs were significantly correlated with FE-biomass ($r=0.56$, $P=0.004$), while no significant correlations were found with SIR-biomass. FE-biomass and SIR-biomass of the 12 forest soils were moderately correlated ($r=0.47$, $P=0.021$). In the soils of the pine forests SIR-biomass was affected differently by environmental factors as compared with the other forest soils. When the pine forest soils were excluded, SIR-biomass g^{-1} organic C was correlated with soil pH ($r=0.92$, $P<0.001$). In Fig. 2, regression of SIR-biomass versus soil pH is presented for spring and autumn data, respectively ($r^2=0.83$, $P<0.001$ and $r^2=0.88$, $P<0.001$). Within the pine forests, SIR-biomass g^{-1} organic C was significantly correlated with the soil moisture content ($r=0.92$, $P<0.001$) and the C-to-N ratio ($r=-0.68$, $P<0.001$). Values of FE-biomass and total PLFAs, when calculated g^{-1} soil organic C, were mainly correlated with the soil NO_3^- content ($r=0.73$, $P<0.001$ and $r=0.80$, $P<0.001$) and with total soil N

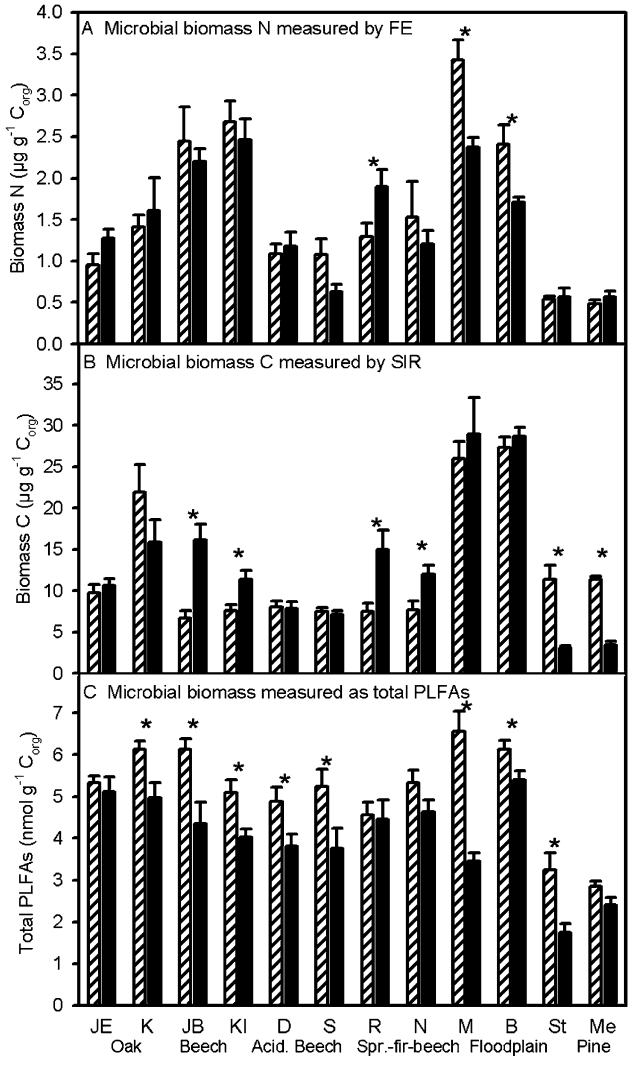

Fig. 1. Comparison of biomass data derived by three different methods: (A) concentration of microbial biomass N measured by chloroform fumigation–extraction, (B) microbial biomass C determined by substrate-induced respiration and (C) concentration of total PLFAs in the 12 forest mineral soils studied. All biomass data are calculated g^{-1} organic C. Hatched bars represent measurements from spring, solid bars represent measurements from autumn. Significant differences in biomass data among both sampling dates are indicated (*). Values are means of 10 subsamples, error bars are standard errors. Abbreviations of study sites are shown in Table 1.

($r=0.73$, $P<0.001$ and $r=0.54$, $P=0.007$). Biomass values calculated g^{-1} soil organic C were generally highest in soils of the floodplain forests. However, high amounts of FE-biomass g^{-1} soil organic C were also found in soils of the woodruff–beech forests. Lowest values of microbial biomass g^{-1} soil organic C were measured in the pine forest soils (Fig. 1).

By using specific PLFAs we distinguished between bacterial and fungal biomass. The use of PLFA 18:2ω6,9 as a marker of fungal biomass was verified by comparing the amount of this fatty acid with the ergosterol content of randomly selected samples ($n=55$), which showed a significant correlation between these two fungal biomarkers ($r=0.94$, $P<0.05$; Fig. 3). The quantity of fungal PLFA 18:2ω6,9 ranged between 3.7 and 31 nmol g^{-1} dry wt, and the ratio of fungal PLFA 18:2ω6,9 to PLFAs indicative of bacterial biomass varied between 0.03 and 0.18 in the different soils.

Calculated g^{-1} soil organic C, bacterial PLFAs were correlated with soil moisture ($r=0.87$, $P<0.001$), soil NO_3^- ($r=0.84$, $P<0.001$), total soil N ($r=0.54$, $P=0.007$) and the concentration of sugars ($r=0.51$, $P=0.011$) and amino acids ($r=0.41$, $P=0.049$) in soil. Fungal PLFA 18:2ω6,9 g^{-1} soil organic C was significantly correlated with the C-to-N ratio ($r=0.63$, $P=0.001$).

3.2. Community composition

Similar results were obtained from measurements of individual PLFAs in the 12 forest soils in spring (Table 5) and in autumn of the same year (data not presented). Principal components analysis (PCA) of the PLFA data from both seasons resulted in very similar patterns. At both times PCA revealed that the PLFA profiles from soils under the floodplain and the pine forests were compositionally distinct from each other and from the profiles of the other forest soils. The floodplain and the pine forests separated along both PC1 and PC2 and they were also strictly separated from the other forest stands (Figs. 4 and 5). By their loading values it is evident that the fatty acids 18:2ω6,9, 18:1ω9 and 10Me18:0 were most important for the separation of the pine forests (Figs. 4 and 5), which in these soils had their highest relative abundances (Table 5). The fungal fatty acid 18:2ω6,9 accounted for 5.8–7.8% of the total PLFAs in the pine stands, while it comprised only between 1.5 and 3.7% in the other forest stands. PLFA 10Me18:0, common in actinomycetes, comprised between 4.1 and 5.0 mol% in the pine forest soils but averaged only 1.4 and 2.1 mol% in soils under the floodplain stands. The floodplain forests had high mole percentages of the unsaturated fatty acids 16:1ω5 and 16:1ω7, the cyclopropane fatty acid 17:0cy, the anteiso-branched a15:0 and a17:0 and the methyl-branched 10 Me16:0 (Table 5).

The scores of the other forest stands were less clearly separated in the PCAs. In the PCA from the autumn measurement, a shift in the PLFA pattern occurred along PC1 from the woodruff–beech stands across the oak and the spruce–fir–beech stands towards the acidophilous beech stands, resulting in the separation of the woodruff–beech from the acidophilous beech stands (Fig. 5).

The first two components of the PCAs explained 42 and 44% of the variation in the PLFA-data from spring and autumn, respectively. In spring, the first component of the PCA appeared to reflect differences in soil pH ($r^2=0.72$, $P<0.001$; linear regression of scores for PC1 versus soil pH) and in SIR-biomass ($r^2=0.42$, $P<0.001$). In addition, the scores for PC1 were significantly correlated

Table 3
Soil chemical properties of the forest stands under study measured in spring

Forest type	Site	Soil moisture (%)	pH	Total N (%)	C_{org} (%)	C-to-N	NO_3–N (μg g^{-1} dry wt)	Sugars (μg g^{-1} dry wt)	Amino acids (μg g^{-1} dry wt)
Oak–Hornbeam	JE	34.2±0.7	4.5±0.2	0.22±0.01	5.04±0.34	23.4±0.6	125.5±3.9	111.6±14.2	5.8±0.8
	K	29.8±0.6	5.4±0.3	0.20±0.01	4.23±0.21	21.0±0.4	121.5±1.0	118.4±9.5	9.2±1.4
Woodruff–beech	JB	35.4±0.9	5.1±0.3	0.19±0.01	4.38±0.31	22.5±0.8	131.8±2.3	72.9±7.1	7.3±1.6
	Kl	34.3±1.2	4.1±0.0	0.33±0.02	4.36±0.32	13.1±0.4	123.1±9.0	66.5±10.7	5.8±1.2
Acidophilous	D	40.9±2.7	4.6±0.2	0.35±0.04	9.45±1.34	26.9±0.7	25.1±3.5	168.3±27.7	19.4±4.6
beech	S	32.0±1.2	4.0±0.0	0.30±0.02	7.03±0.56	23.5±0.5	0.6±0.2	130.6±10.5	11.5±1.2
Spruce–fir–beech	R	57.9±3.2	4.9±0.4	0.94±0.15	16.00±2.38	17.1±1.1	340.7±59.4	255.5±42.1	26.8±8.5
	N	43.3±1.2	4.0±0.1	0.38±0.03	6.46±0.63	16.9±0.7	216.8±19.7	93.5±6.1	5.6±1.2
Floodplain	M	38.7±0.8	7.2±0.0	0.47±0.02	5.46±0.34	11.7±0.4	247.3±27.5	110.7±4.0	30.0±1.0
	B	29.4±0.7	7.4±0.0	0.23±0.01	3.92±0.13	17.2±0.5	202.0±8.9	57.5±3.5	14.2±2.5
Pine	St	35.4±3.1	7.4±0.1	0.61±0.09	16.99±2.69	28.0±1.0	4.6±0.9	185.4±20.3	56.7±4.6
	Me	22.7±0.6	7.4±0.0	0.26±0.02	9.64±0.63	37.0±2.0	2.3±0.6	122.9±26.2	31.3±7.0

Values represent means of 10 subsamples ±SE. For abbreviations of study sites see Table 1.

to the mean annual temperature ($r^2=0.40$, $P=0.027$). PC1 was also correlated to the content of NH_4^+ ($r^2=0.24$, $P=0.001$) in the soils. The second component of the PCA like PC1 was correlated to the soil pH ($r^2=0.16$, $P=0.007$) but seemed mainly to reflect differences among the soils in the C-to-N-ratio ($r^2=0.65$, $P=0.000$) and the ratio of fungal to bacterial PLFAs ($r^2=0.69$, $P=0.000$). Furthermore, PC2 was correlated to FE-biomass ($r^2=0.24$, $P=0.001$) and total PLFAs ($r^2=0.26$, $P<0.001$) as well as to the content of soil organic C ($r^2=0.22$, $P=0.001$) in the soils. In autumn, both principal components were again correlated to the soil pH ($r^2=0.36$, $P=0.038$ and $r^2=0.47$, $P=0.014$) and, in addition, were both correlated to the ratio of fungal to bacterial PLFAs ($r^2=0.61$, $P=0.001$ and $r^2=0.47$, $P=0.007$). Scores for PC1 were correlated to SIR- ($r^2=0.73$, $P<0.001$) and FE-biomass ($r^2=0.39$, $P=0.029$). Furthermore, there was a correlation of PC1 with the mean annual temperature ($r^2=0.34$, $P=0.047$). PC2 appeared to reflect the C-to-N ratios ($r^2=0.84$, $P<0.001$), as in spring, and was also correlated to total PLFAs ($r^2=0.59$, $P=0.003$).

4. Discussion

4.1. Microbial biomass in natural forest soils

Both quantitative and qualitative information on the soil microbial communities of the 12 forests was provided by measurement of microbial biomass and analysis of microbial community composition. The size of the microbial biomass was shown to be tightly coupled with the content of SOM, which is the case in most soils because they are commonly resource limited (Wardle, 1992). The biomass data which were obtained by three independent methods are complementary because they provide different types of information about the microbial community. FE-biomass assesses the chloroform-susceptible biomass (Wardle and Parkinson, 1990) and gives a measure of the labile N pool represented by the microorganisms (Martens, 1995). Differences in FE-biomass between spring and autumn sampling may reflect differences in soil N-availability. In the floodplain forest soils, lower soil nitrate content in autumn relative to spring corresponded with

Table 4
Soil chemical properties of the forest stands under study measured in autumn

Forest type	Site	Soil moisture (%)	pH	Total N (%)	C_{org} (%)	C-to-N	NO_3–N (μg g^{-1} dry wt)	Sugars (μg g^{-1} dry wt)	Amino acids (μg g^{-1} dry wt)
Oak–Hornbeam	JE	20.1±0.8	5.0±0.1	0.34±0.03	5.60±0.56	16.1±0.5	38.5±6.3	114.3±3.4	2.5±0.3
	K	16.6±1.1	5.7±0.3	0.26±0.02	4.93±0.28	18.9±0.5	17.7±4.9	97.6±3.0	2.2±0.3
Woodruff–beech	JB	21.3±1.0	5.0±0.3	0.27±0.02	4.35±0.35	16.1±0.6	25.2±8.3	75.4±1.5	2.4±0.4
	Kl	25.3±0.8	4.4±0.1	0.24±0.01	5.11±0.26	21.4±1.5	40.5±11.4	97.5±4.9	1.5±0.1
Acidophilous	D	17.4±2.0	4.9±0.2	0.42±0.07	8.50±1.11	21.1±0.9	0.8±0.3	178.0±17.4	2.7±1.0
beech	S	16.3±1.3	3.9±0.0	0.31±0.03	7.00±0.70	22.5±0.8	0.4±0.1	118.6±7.3	3.5±0.5
Spruce–fir–beech	R	60.5±3.2	5.2±0.3	0.88±0.12	21.44±2.98	24.6±1.6	220.2±60.4	251.6±39.8	15.5±3.2
	N	45.7±1.1	4.1±0.2	0.42±0.03	9.90±0.84	23.4±0.7	92.2±10.0	170.5±21.2	3.5±0.2
Floodplain	M	27.2±0.9	7.4±0.0	0.34±0.01	5.61±0.20	16.7±0.3	135.0±15.1	192.0±8.1	10.2±1.5
	B	29.6±1.0	7.3±0.0	0.31±0.02	4.34±0.24	14.3±0.3	132.0±12.2	94.0±3.1	11.5±0.8
Pine	St	14.4±2.5	7.5±0.1	0.43±0.08	17.73±1.37	57.1±10.6	0.5±0.1	123.6±12.4	9.5±0.9
	Me	10.5±0.7	7.6±0.0	0.21±0.02	10.67±0.70	52.8±4.5	0.4±0.0	157.8±10.1	9.6±0.7

Values represent means of 10 subsamples ±s.e. For abbreviations of study sites see Table 1.

Table 5
PLFAs in soils under forest stands representing different forest types measured in spring, expressed as %total PLFAs ($n=10$)

PLFA	Percent total PLFA composition (mean ± SE)											
	Oak		Beech		Acidophilous beech		Spruce–fir–beech		Floodplain		Pine	
	JE	K	JB	Kl	D	S	R	N	M	B	St	Me
14:0***	1.26 ± 0.15	1.25 ± 0.14	0.40 ± 0.16	0.00 ± 0.00	1.45 ± 0.03	0.67 ± 0.19	1.30 ± 0.06	1.17 ± 0.14	1.18 ± 0.06	1.34 ± 0.03	2.02 ± 0.12	1.61 ± 0.10
i15:0***	8.41 ± 0.22	7.53 ± 0.11	7.77 ± 0.36	6.85 ± 0.23	8.18 ± 0.24	7.75 ± 0.35	8.77 ± 0.35	8.61 ± 0.23	7.70 ± 0.32	9.33 ± 0.23	4.99 ± 0.09	3.91 ± 0.08
a15:0***	5.41 ± 0.38	5.99 ± 0.45	6.37 ± 0.30	6.91 ± 0.33	4.44 ± 0.21	3.20 ± 0.28	5.51 ± 0.38	4.56 ± 0.53	9.90 ± 3.53	6.98 ± 0.13	3.89 ± 0.11	3.46 ± 0.06
15:0**	0.39 ± 0.16	0.60 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.81 ± 0.20	0.54 ± 0.09	0.48 ± 0.17	0.06 ± 0.06	0.29 ± 0.12	0.85 ± 0.10	0.80 ± 0.02
i16:0***	6.13 ± 0.45	5.88 ± 0.19	4.70 ± 0.22	4.61 ± 0.10	6.19 ± 0.27	7.12 ± 0.53	3.76 ± 0.16	4.10 ± 0.36	3.78 ± 0.16	3.71 ± 0.05	4.08 ± 0.14	3.96 ± 0.13
16:1ω7***	6.53 ± 0.22	7.55 ± 0.27	7.74 ± 0.50	5.91 ± 0.15	7.40 ± 0.84	7.22 ± 0.20	7.26 ± 0.34	6.84 ± 0.22	8.82 ± 0.46	9.48 ± 0.21	8.53 ± 0.23	8.75 ± 0.17
16:1ω5***	2.92 ± 0.27	3.75 ± 0.22	4.34 ± 0.41	2.61 ± 0.31	3.52 ± 0.21	2.31 ± 0.08	3.01 ± 0.33	3.35 ± 0.21	5.29 ± 0.25	6.02 ± 0.15	3.08 ± 0.11	3.18 ± 0.08
16:0***	12.9 ± 0.35	12.3 ± 0.24	12.1 ± 0.34	11.6 ± 0.17	13.9 ± 0.51	12.8 ± 0.50	11.5 ± 0.24	11.2 ± 0.22	10.5 ± 0.43	11.3 ± 0.08	12.7 ± 0.22	12.8 ± 0.20
10Me16:0***	7.32 ± 0.34	3.44 ± 0.93	7.00 ± 0.39	6.86 ± 0.21	6.81 ± 0.24	9.70 ± 0.51	7.46 ± 0.22	7.12 ± 0.24	9.59 ± 0.44	10.1 ± 0.39	5.01 ± 0.24	4.44 ± 0.16
i17:0***	1.86 ± 0.08	1.81 ± 0.05	1.94 ± 0.06	2.00 ± 0.10	1.51 ± 0.05	2.15 ± 0.68	1.71 ± 0.05	2.06 ± 0.31	2.03 ± 0.09	2.29 ± 0.06	1.16 ± 0.13	1.26 ± 0.04
a17:0***	1.76 ± 0.06	2.24 ± 0.14	1.97 ± 0.08	1.79 ± 0.20	1.55 ± 0.07	0.95 ± 0.18	1.63 ± 0.05	1.69 ± 0.05	2.22 ± 0.09	2.46 ± 0.06	2.23 ± 0.04	2.25 ± 0.07
17:0cy*	2.00 ± 0.06	2.11 ± 0.10	1.96 ± 0.13	1.79 ± 0.20	2.45 ± 0.08	1.24 ± 0.15	2.09 ± 0.11	1.63 ± 0.13	2.07 ± 0.08	2.27 ± 0.04	1.74 ± 0.06	1.73 ± 0.04
17:0***	0.06 ± 0.06	0.21 ± 0.21	0.00 ± 0.00	0.00 ± 0.00	0.78 ± 0.09	0.22 ± 0.12	0.17 ± 0.07	0.05 ± 0.05	0.06 ± 0.06	0.19 ± 0.09	0.46 ± 0.08	0.56 ± 0.06
10Me17:0***	1.75 ± 0.14	1.39 ± 0.08	1.15 ± 0.15	1.22 ± 0.14	1.22 ± 0.05	2.66 ± 0.72	0.94 ± 0.12	1.49 ± 0.32	0.00 ± 0.00	0.00 ± 0.00	0.64 ± 0.11	0.77 ± 0.03
18:2ω6,9***	2.26 ± 0.13	2.59 ± 0.07	2.68 ± 0.26	1.55 ± 0.22	3.50 ± 0.24	3.65 ± 0.27	1.76 ± 0.11	1.49 ± 0.19	1.96 ± 0.25	1.55 ± 0.09	6.35 ± 0.45	7.84 ± 0.32
18:1ω9***	6.42 ± 0.25	7.53 ± 0.32	7.35 ± 0.32	6.64 ± 0.24	6.67 ± 0.18	7.44 ± 0.22	6.52 ± 0.21	6.41 ± 0.21	7.51 ± 0.31	7.09 ± 0.70	9.15 ± 0.77	10.4 ± 0.11
18:1ω7***	10.1 ± 1.24	12.2 ± 0.41	14.3 ± 0.85	14.4 ± 0.23	12.4 ± 0.76	12.6 ± 0.70	14.2 ± 0.44	12.3 ± 0.63	12.5 ± 0.64	10.0 ± 0.32	14.6 ± 0.43	13.5 ± 0.19
18:1ω5***	1.96 ± 0.23	2.49 ± 0.15	1.80 ± 0.12	1.32 ± 0.29	0.62 ± 0.11	0.64 ± 0.18	1.84 ± 0.12	1.27 ± 0.10	1.82 ± 0.12	1.67 ± 0.07	1.65 ± 0.19	1.30 ± 0.10
18:0***	2.56 ± 0.03	2.46 ± 0.05	2.45 ± 0.30	2.59 ± 0.06	2.98 ± 0.14	2.81 ± 0.08	2.63 ± 0.09	2.53 ± 0.09	2.15 ± 0.10	2.36 ± 0.07	2.32 ± 0.14	2.46 ± 0.05
10Me18:0***	2.98 ± 0.16	3.45 ± 0.20	2.80 ± 0.15	2.27 ± 0.28	2.05 ± 0.10	2.24 ± 0.10	1.90 ± 0.20	2.33 ± 0.21	1.69 ± 0.12	1.40 ± 0.10	4.78 ± 0.30	4.14 ± 0.29
19:0cy***	1.24 ± 0.08	0.91 ± 0.11	0.44 ± 0.15	1.66 ± 0.12	0.43 ± 0.27	2.32 ± 0.22	2.16 ± 0.18	3.19 ± 0.26	0.12 ± 0.08	0.05 ± 0.05	0.67 ± 0.08	0.89 ± 0.05
19:1ω8***	5.38 ± 0.18	4.49 ± 0.24	5.69 ± 0.23	7.63 ± 0.18	4.94 ± 0.33	6.92 ± 0.33	7.39 ± 0.68	7.68 ± 0.53	2.83 ± 0.14	2.12 ± 0.07	3.37 ± 0.18	2.65 ± 0.10
20:0***	0.50 ± 0.14	0.38 ± 0.13	0.06 ± 0.06	0.00 ± 0.00	1.38 ± 0.27	0.88 ± 0.11	0.78 ± 0.04	1.13 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.8 ± 0.09	0.93 ± 0.03
Total PLFAs[a]	268.55	257.59	264.04	221.53	464.44	356.34	698.97	335.93	352.06	242.17	478.27	269.57

Significant differences in individual PLFAs among forest types are indicated by ***$P<0.001$, **$P<0.01$ and *$P<0.05$, respectively. For abbreviations of study sites see Table 1.

[a] Total amounts of PLFAs in nmol g^{-1} dry wt.

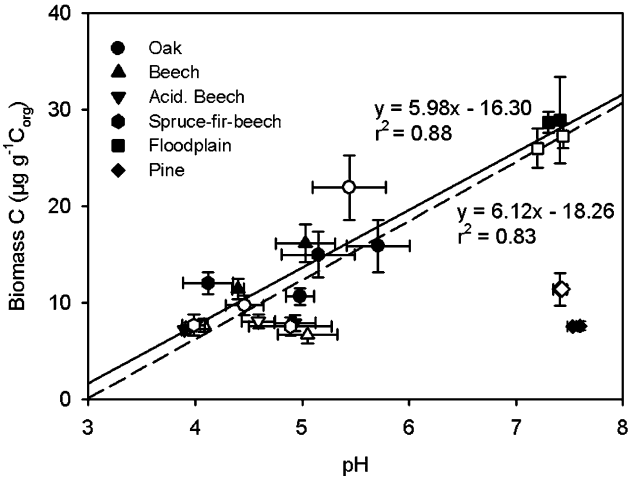

Fig. 2. Correlation between microbial SIR-biomass (biomass C g^{-1} organic C) and pH in the 12 mineral forest soils. The same symbols are used for the different forest types for spring (open symbols) and autumn data (filled symbols) and represent means of 10 subsamples; error bars are standard errors. Regression lines and equations are shown for spring (dashed line) and autumn (solid line). The pine forests are not included in the regressions.

lower values of FE-biomass while no decrease in SIR-biomass was measured.

The PLFA analysis presumably shows the maximum amount of microbial biomass present in soil (Frostegård and Bååth, 1996). It applies to the living microbial cells because the phospholipids decompose rapidly to diglycerides following cell death (White et al., 1979). FE- and PLFA-biomass appeared to be interrelated when the 12 forest soils were compared and they were both primarily correlated with the soil N content, which confirms the view that FE-biomass represents a subset of the total microbial biomass. A good linear relationship between microbial biomass measured by FE and total PLFAs has been observed by Bailey et al. (2002), who proposed an equation describing the prediction of FE-biomass with PLFAs.

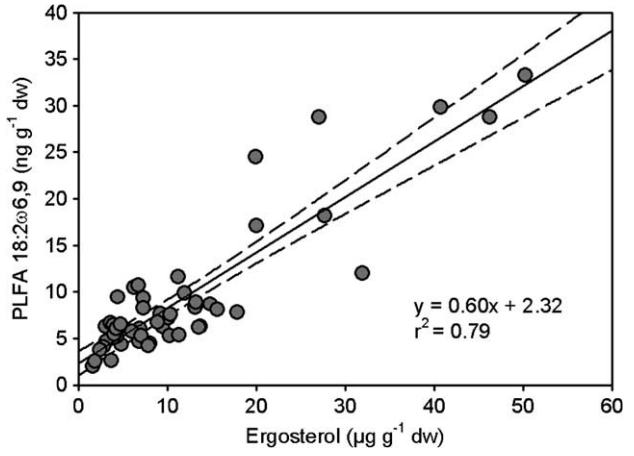

Fig. 3. Correlation between the content of ergosterol (µg g^{-1} dry wt) and the fatty acid 18:2ω6,9 (nmol g^{-1} dry wt) in a group of 54 subsamples. Regression line (solid line) and confidence intervals (dashed lines) are shown.

While SIR-biomass in the 12 forest soils was found to be moderately correlated to FE-biomass, it showed no correlation with total PLFAs. This may be indicative of the fact that SIR measures the glucose-responsive subset of the microbial community (Wardle and Ghani, 1995), whereas PLFAs are extracted from all cells regardless of their response to substrate-amendment. Wardle and Parkinson (1990) have argued that different organisms may respond differently to added C-substrates in SIR, which may weaken the relationship between biomass data derived by SIR and other methods. This may be of special relevance if soils are compared which vary in microbial community composition.

SIR-biomass applies to the metabolically active subset of the microbial community, which in all but the pine forest soils appeared to be strongly affected by the soil pH (Fig. 2). An increase in the ratio of microbial biomass C to soil organic C in forest soils with an increase in soil pH has been reported and may reflect microbial community stress under conditions of low soil pH (Anderson and Domsch, 1993). Low soil pH has been identified as the major constraint on the soil microorganisms within the study sites of the oak, acidophilous beech and spruce–fir–beech forests (Hackl et al., 2000). The pine forest soils, in contrast, are high in pH as a consequence of the geological substrate, dolomite, and therefore microbial growth or activity in these soils are not limited by low pH values. SIR-biomass g^{-1} organic C probably was low in these soils because constraints other than soil pH were acting on the soil microbial communities. The pine forests were characterised by a low soil moisture content and high C-to-N ratios, which in these soils appeared to determine within site-variability in SIR-biomass. In autumn, when there was exceptionally dry weather, limitation of microbial activity by low soil water content was reflected in low values of SIR-biomass g^{-1} organic C relative to spring. Differences among the seasons in PLFA- and FE-biomass were less pronounced, indicating that the metabolically active subset was more affected by limitation in soil water supply than the total microbial community.

4.2. Microbial community composition in natural forest soils

By the relative contribution of individual PLFAs, it was shown that the microbial communities in the soils of the pine and the floodplain forests showed marked differences in composition relative to each other and to the other forest soils. The pine forest stands were set apart from all other forest stands by a higher relative abundance of three particular PLFAs: fungal PLFA 18:2ω6,9 (Olsson, 1999), PLFA 10Me18:0, common in actinomycetes (Kroppenstedt, 1985), and PLFA 18:1ω9, which is present in most fungal species (Amano et al., 1992). One reason for the high prevalence of fungal PLFA in the pine forest soils may be that fungi are presumably more efficient than bacteria in

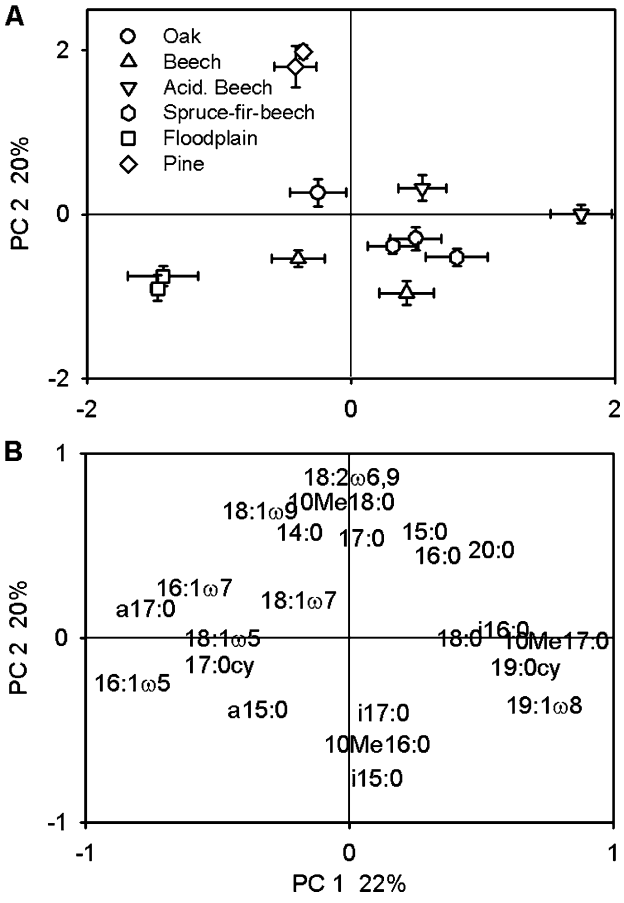

Fig. 4. (A) Score plot of PCA showing the separation of the mineral soil layers of the 12 forest stands along principal components (PC) 1 and 2 and (B) loading values for the PLFAs, measured in spring. PCA was performed using clockwise rotation of the first two components by 45°. Symbols indicating different forest types represent means of 10 subsamples, error bars are standard errors.

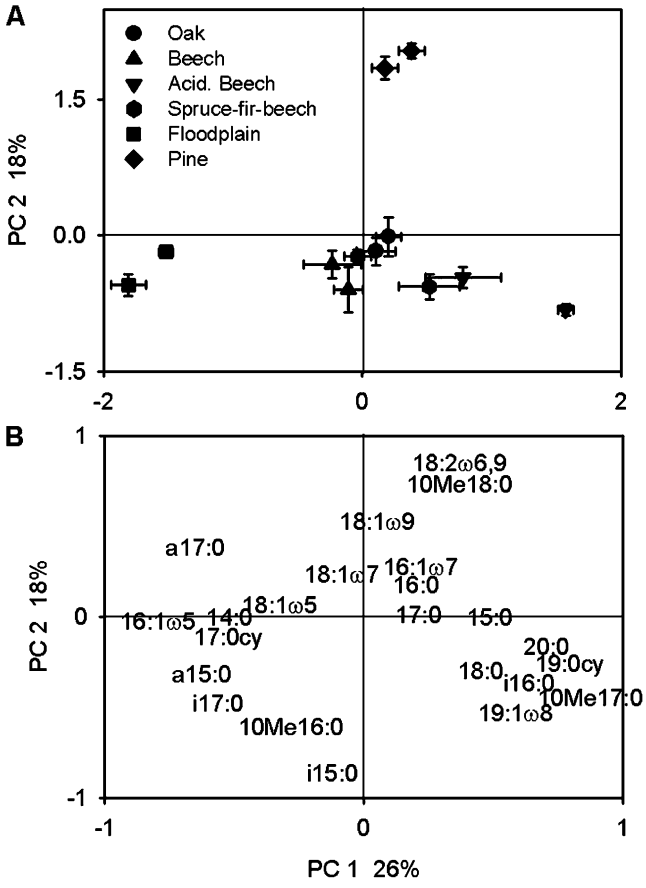

Fig. 5. (A) Score plot of PCA showing the separation of the mineral soils of the 12 forest stands along principal components (PC) 1 and 2 and (B) loading values for the PLFAs, measured in autumn. Symbols indicating different forest types represent means of 10 subsamples, error bars are standard errors.

coping with the degradation of pine litter. Pine litter contains high amounts of recalcitrant polymeric phenolic compounds such as lignin and tannin. It is well known that fungi are the organisms principally responsible for lignin degradation (Dix and Webster, 1995). Part of the fungal PLFA 18:2ω6,9 in the pine forest soils may be derived from ectomycorrhizal fungi (Olsson, 1999), which are known to contribute substantially to the fungal biomass in coniferous forests (Finlay and Söderström, 1989). Ectomycorrhizal fungi have been reported to be dominant colonisers of species within the Pinaceae family (Smith and Read, 1997). High abundance of PLFA 10Me18:0, indicating actinomycetes, may be related to the high soil pH of the pine forests, since an increase in this fatty acid has also been observed after the liming of forest soils (Frostegård et al., 1993).

The PLFAs most prevalent in the floodplain forests are representative of heterogeneous groups of microorganisms, comprising 16:1ω7 and 17:0cy, common in Gram-negative bacteria (Harwood and Russel, 1984), the anteisobranched a15:0 and a17:0, typically present in Gram-positive bacteria (Harwood and Russel, 1984), the unsaturated 16:1ω5, which is typical of VA-mycorrhizal fungi (Olsson, 1999) but is also present in Gram-negative bacteria (Nichols et al., 1986), and the methylbranched 10Me16:0, which is an indicator for sulfate reducing bacteria (Rajendran et al., 1992) but has also been ascribed to other bacterial genera (O'Leary and Wilkinson, 1988) and to actinomycetes (Kroppenstedt, 1985). There are some indications that PLFA 16:1ω5 may have been derived from VA-mycorrhizal fungi: the main tree species of the floodplain forests are of the genera *Fraxinus*, *Populus*, *Acer* and *Alnus*, which on nutrient-rich soils are known to be either always or facultatively VA-mycorrhizal. Furthermore, these tree species often have extensive VA-mycorrhizal understories (Smith and Read, 1997). It has also been suggested that PLFA 16:1ω5 may be indicative of organisms which respond to changes in easily available C in soil (Frostegård et al., 1996; Priha et al., 1999). Thus, this PLFA may indicate the presence of microorganisms which readily adjust to variations in the soil C status due to inputs of allochthonous material at occasions when these soils are flooded.

Our results suggest that the microbial communities in the soils of the floodplain forests diverged from those of the other deciduous and mixed forest stands because they responded to the higher pH value of these soils. The fatty acids a15:0, a17:0, 17:0cy and 16:1ω5, which were present in high mole percentages in the floodplain forests, usually increase when pH increasing measures such as liming are applied to forest soils (Frostegård et al., 1993; Bååth et al., 1995). A pH dependency of the composition of the microbial community has been demonstrated experimentally: by incubating soils with ^{13}C-acetate at different soil pH, Arao (1999) found that at low pH ^{13}C was mainly incorporated into 18:2ω6,9, 16:0 and 18:1ω9 and that ^{13}C was low or not detectable in many bacterial PLFAs, suggesting that the growth of bacteria was inhibited. Raising soil pH to 7 or 8 resulted in ^{13}C incorporation into PLFAs representative of bacteria, possibly because bacterial growth was facilitated. Since the microbial communities at their natural sites are exposed to many environmental factors which are interrelated, their reaction to specific site conditions certainly differs from a response to an artificial change in pH. Nevertheless, our results correspond with the amendment studies in that they reveal a higher relative abundance of many bacterial PLFAs (a15:0, i17:0, 17:0cy, 16:1ω5, 16:1ω7) in the floodplain forests versus the more acidic oak, beech and spruce–fir–beech forests.

The composition of the soil microbial communities in the pine forests differed markedly from that in the floodplain forests, although the soil pH was similar for both forest types. The high prevalence of PLFAs which are typical of fungi in the pine forests may be related to the high C-to-N ratio and the low water content of the soils. A higher proportion of fungi has been observed in forest soils with a high versus a low C-to-N ratio (Pennanen et al., 1999). Soils of the pine forests were stony and restricted to a small layer and thus were prone to desiccation. We suppose that these conditions are more easily dealt with by fungi, which are considered more drought-tolerant and less dependent on a well-balanced nutrient supply than bacteria. In particular, the capacity of fungi to translocate N to suitable C sources via hyphae is thought to be important in coniferous forests with a high C-to-N ratio (Clarholm, 1994). This view is supported by the fact that the amount of bacterial but not fungal biomass in the 12 forest soils was mainly correlated with available N. The abundance of fungal PLFA 18:2ω6,9 in the 12 forest soils, indeed, was positively correlated to the soil C-to-N ratio.

As compared with the floodplain and pine forest soils, which are azonal vegetation types, the microbial communities proved to be more similar in soils from the oak, beech and spruce–fir–beech forests. These forests represent the zonal forest types of the region, and the different plant communities reflect gradients in the regional climate (Ellenberg, 1996). Differences between the forest stands in microbial community composition likewise appeared to correspond with differences among the sites in the mean annual temperature. As the soils of these sites do not show any extreme properties such as extreme water conditions or strong nutrient limitation, they probably offer more similar environmental conditions for the soil microorganisms as compared with the floodplain and pine forest soils. Differences in microbial community composition among the 12 forest soils studied were correlated with differences in soil pH and C-to-N ratio and, to a lesser extent, with differences in soil nitrate and microbial biomass content. Differences in these qualities were less pronounced within the zonal forest types and may therefore have resulted in lower discrimination of the microbial communities as compared with the pine and floodplain forests, where more extreme environmental conditions prevail.

Our study suggests that the occurrence of azonal forest types at sites with specific soil conditions is accompanied by the development of distinct soil microbial communities. In the floodplain forests, soil pH seemed to be responsible for the discrimination of the soil microbial communities against those of the zonal forest types. The microbial communities in the pine forest soils, however, appeared to be set apart from those of the other forest types by a higher abundance of actinomycetes and a high prevalence of fungi, which may be better able to cope with nitrogen and water supply limitations.

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