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Spatial variation and patterns of soil microbial community structure in a mixed spruce–birch stand

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Abstract

To explore the spatial variation of the soil microbial community within a mixed Norway spruce–birch stand, and to test if the spatial patterns of the microbial community are related to the position of trees, we sampled the forest floor at two spatial scales and used the phospholipid fatty acid (PLFA) patterns as indicators of the microbial community structure. Of the 32 most common PLFAs, 20 (62%) were clearly spatially autocorrelated, and the limit of spatial dependence (range) varied between 1 m and 11 m. The variation in the community structure was examined by subjecting the PLFAs to a principal component analysis. The first two principal components described variation structured at two different spatial scales. The range of the microbial community for the first component was 4.6 m, whereas for the second component it was only 1.5 m. The microbial community was influenced by the position of the trees. Spruce trees had a much stronger influence on PLFA patterns than birch trees, and the first principal component, as well as 12 PLFAs, was influenced by spruce trees. Several branched PLFAs, characteristic of Gram-positive bacteria, loaded negatively on the second principal component. These PLFAs represent a complex of associated microorganisms that aggregated in small patches away from birch trees. A comparison with a laboratory experiment suggests that although the tree species differ in their influence on soil moisture and ground vegetation, their influence on the microbial community were, to a large extent, connected to the quality of soil organic matter associated with the two trees.

Keywords: Spatial variation; Soil microbial community; Phospholipid fatty acids; Tree influence; *Picea abies*; *Betula pubescens*

1. Introduction

Organisms are not distributed uniformly in the environment, rather the abundance and activity of organisms change along environmental gradients. Biotic processes often result in aggregation of organisms even within homogenous environments (Legendre and Fortin, 1989). Spatial aggregation can easily be observed in some organisms (e.g., plants or sessile animals), whereas the aggregations of inconspicuous organisms (e.g., soil organisms) may be less obvious. The phospholipid fatty acid (PLFA) or fatty acid methyl ester (FAME) in conjunction with geostatistics can be used to reveal the spatial structure of the variation in microbial communities. The latter technique was used by Cavigelli et al. (1995). For example the range and the degree of spatial dependence can be estimated, which together provide information of the scale at which communities aggregate. Furthermore, the information on the spatial autocorrelation of a variable can be used to interpolate the values between sample points (Robertson, 1987).

Physical and chemical characteristics of soil are often spatially dependent (i.e., aggregated) at scales that range from decimetres to several hundred metres and spatial dependence can occur simultaneously at

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different scales (Oliver and Webster, 1987; Robertson et al., 1988; Bringmark, 1989; Jackson and Caldwell, 1993; Schlesinger et al., 1996; Robertson et al., 1997; Möttönen et al., 1999). However, the spatial structure of microbial communities and microbial activity have received less attention, presumably because the number of samples needed to characterise the spatial variation for a given site is large. Studies conducted in agricultural and shrub–steppe ecosystems suggest that microbial biomass and activity may be spatially dependent at scales <1 m, nested within a larger scale related to variation at the landscape level (Rochette et al., 1991; Smith et al., 1994; Robertson et al., 1997; Smith, personal communication). To our knowledge, there is only one study on the spatial variation of microbial community structure in soil (Cavigelli et al., 1995). This study suggested that the spatial structure in the variation of microbial communities in a cultivated field was at the scale of decimetres or less, i.e. at scales of individual soil aggregates or of the rhizosphere of individual plants.

The microbial communities in forest soils, rich in organic matter, may differ considerably from those found in grassland and in arable soils with lower organic matter content (Frostegård and Bååth, 1996). In forest ecosystems, trees may cause soil resource heterogeneity and spatial patterns in soil properties that correspond to the positions of trees (Boerner and Koslowsky, 1989; Boettcher and Kalisz, 1990; Kleb and Wilson, 1997). For example, microbial biomass and activity in a mixed forest were spatially dependent at distances up to 8 m, and birch and spruce trees differed in their influence on soil biological properties (Saetre, 1999). In a Scots pine forest fungal biomass aggregated on a similar scale, with a range of spatial dependence of 4 m (Möttönen et al., 1999). Thus, it can be expected that fatty acids other than those spatially structured in arable soils will be spatially structured in forest soils, and that the variation in the microbial communities will be spatially structured at a larger spatial scale in forest soils, corresponding to the position of trees in the forest.

Our aim was to explore spatial variation of the soil microbial community on a scale from 0.2 to 20 m within a mixed Norway spruce–birch stand, where the spatial patterns of ground vegetation, soil microbial biomass, and activity have earlier been described (Saetre, 1999). To do this, we sampled soil at two spatial scales and used PLFA patterns as indicators of the microbial community structure. We applied geostatistics to characterise the spatial structure of the variation in single PLFAs and total PLFA pattern. Furthermore, we tested the hypothesis that the spatial variation in the PLFAs was related to the position of spruce and birch trees in the stand, by using a simplified version of the ecological field theory (Wu et al., 1985).

2. Materials and methods

2.1. Study site

The study site is located in a 55 year-old mixed stand of Norway spruce (*Picea abies*) and birch (*Betula pubescens*) near Månkarbo (60°14′ N, 17°35′ E), in central Sweden. The site is 40 m above sea level, the temperature sum of the growing season (above 5°C) is 1310 degree-days, and average precipitation is 400 mm during the growing season. The 7.1 ha stand was a fen used for haymaking before 1942, when it was drained and planted with Norway spruce. Today it is a mixture of planted spruce and self-propagated broad-leaved trees, mainly birch. There are approximately 800 stems ha^{-1}, and the mean tree height is 18 m. The study area is a 14×22 m rectangle in the centre of the stand with 35 m to the nearest border. The area was chosen to be level and topographically uniform to avoid anisotropic effects in the geostatistical analysis. The soil is a Histosol, approximately 30 cm thick, overlying fluvial clay. The ground vegetation was dominated by the herb *Oxalis acetosella* and the moss *Brachytecium reflexum*. Other common species were the herbs *Maianthemum bifolium*, *Trientalis europea*, *Viola epipsila,* and *Rubus saxatilis* and the mosses *Pleurozium schreberi* and *Dicranum scoparium*.

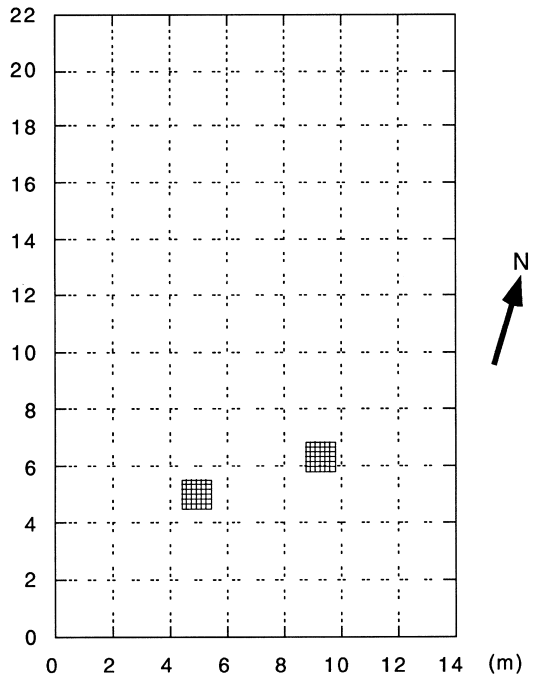

Fig. 1. The soil sampling scheme in a mixed Norway spruce–birch stand. Soil was sampled at each grid node in the 2 m and the two 0.2 m grids, respectively.

2.2. Soil sampling and analyses

In September 1996 we sampled the soil in our study area at the nodes of a 2 × 2 m grid (Fig. 1). In addition to these 96 points, we sampled a further 80 points at the nodes of a 0.2 × 0.2 m grid within each of two 1.2 m × 1.2 m squares. The location of the two small grids were randomly chosen within the study area, with the limitation that one grid should represent an area influenced by spruce ($IP_{spruce} \geq 30$, see below for explanation) and the other grid an area influenced by birch ($IP_{birch} \geq 30$). After removing the litter, two 4.5 cm diameter and 5 cm deep soil cores were taken within a few centimetres of one another, at each grid node. If a tree or a stump stood on the grid node, the sampling position was moved to a location without such structures as close to the original grid node as possible, and the exact spatial position was recorded. The two cores were combined to form a composite sample. The soil was sieved (4 mm), and visible roots were removed by hand on the day of sampling. Samples were stored at 4°C overnight. The next day all samples were frozen for later analysis.

PLFAs were extracted and analysed by the method of Frostegård et al. (1993a). In this study, 8 g soil (fresh weight) was frozen in liquid N_2, freeze-dried and milled. PLFAs were then extracted from a 0.50 g (dry weight) subsample. Methyl esters derived from the phospholipids were analysed on a gas chromatograph (Hewlett Packard 5890, H. P. Company, Avondale, PA, USA) equipped with a flame ionisation detector as described by Frostegård et al. (1993a). Thirty-two fatty acids were identified using the retention times determined for soil PLFAs by gas chromatography–mass spectrometry (Frostegård et al., 1993b). PLFAs considered to be of bacterial origin only (Frostegård et al., 1993b) were summed to give an index of the proportion of bacterial biomass.

Nomenclature of fatty acids follows that used by Tunlid and White (1992). Fatty acids are designated as the total number of carbon atoms, number of double bonds, followed by the position of the double bond from the methyl end of the molecule. *Cis* and *trans* configurations are indicated by *c* and *t*, respectively. Anteiso- and iso-branching are indicated by the prefixes a and i, br indicates unknown methyl branching position, 10Me indicates a methyl group on the 10th carbon atom from the carboxyl end of the molecule, and cy refers to cyclopropane fatty acids. x5 is an unidentified PLFA.

2.3. Statistical analyses

To describe patterns in microbial community structure and their spatial variation, PLFA composition was analysed using principal components analysis (PCA). Each sample was represented by a vector of PLFAs (expressed as the logarithmic transformed mol percent of total PLFA content). The sample scores along the first and second principal components were used as derived variables in further statistical analyses.

We performed univariate geostatistical analysis on 32 PLFAs (expressed as mol percentage), on bacterial PLFA content, and on the sample scores along the first two principal components. Omnidirectional semivariances were calculated with the program MGAP (1993). The semivariances were determined for lag classes (average distance) 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 m for samples taken from the 0.2 m grid. For samples from the 2.0 m grid the classes were 2.0, 4.0, 6.0, 8.0 and 10 m. The semivariance for each class was computed from at least 112 pairs of comparisons.

Model variograms were fitted by least square approximation to the experimental semivariances combining results from both grids, using the NONLIN module in SYSTAT (1992). We used both the spherical and the exponential model with nugget variance. These two models are conditional negative semi-definite, i.e. they are authorised for use in two dimensions (Webster and Oliver, 1990). The two variogram models describe how the semivariance increases monotonically with increasing lag distance until it reaches a maximum at which it levels out. The maximum semivariance is known as the sill ($C_0 + C$). The lag distance at which the sill is reached is known as the range and it marks the limit of spatial dependence. In the exponential model the semivariance approaches its maximum asymptotically and the range is therefore defined as the distance where the semivariance equals 95% of the sill. The intercept of the model variogram is known as the nugget (C_0). The nugget variance represents variation that cannot be resolved with the sampling effort used. Nugget variance usually arises from errors of measurement and from spatial variation at distances smaller than the smallest sampling interval (Webster and Oliver, 1990).

For most variables the spherical model gave the best fit (smallest residual sum of squares). When the two models gave similar fit, we chose the model parameters from the spherical model. This was done to simplify the comparisons of model parameters between individual PLFAs and also because the range of a spherical variogram can be interpreted as the average size of patches, i.e. areas with large or small values. We used the proportion of sill ($C_0 + C$) explained by the structural variance C as a normalised measure of spatial structure. When spatial dependence is strong, this proportion approaches 1, whereas it approaches 0 when all semivariance is accounted for by the nugget variance.

When analysing the spatial structure of variation with variogram it is assumed that no spatial drift is

present in the data. The presence of spatial trends in PLFAs and principal components was examined using a second order polynomial of x and y coordinates, and by visual examination of column and row means. We did not find any evidence for spatial trends in the data.

To present the spatial patterns of the PLFA composition visually, values between actual measurements were estimated by kriging, a geostatistical method that takes full account of a variable's spatial autocorrelation (Webster and Oliver, 1990). We used block kriging to estimate variable values at the nodes of a 0.5 × 0.5 m grid (MGAP, 1993). A minimum of 25 neighboring points were used for each estimate. The 0.5 × 0.5 m grid was chosen to simplify comparisons with previous results on plant cover and light from the site (Saetre, 1999). Maps were produced by contouring in MacGRIDZO (1991).

We used the concept of tree influence potential (IP) as an index of the combined influence of all trees of a canopy species in a spatial location to analyse whether tree species influence the microbial community in a mixed stand. The approach we have used is a simplified version of the ecological field theory described by Wu et al. (1985), and it is discussed in more detail by Saetre (1999). In the present study, IP is an index of the combined influence of all trees within a distance r' at a spatial location p.

$$\mathrm{IP}(p) = \sum_k \mathrm{DBH}_k \exp(-c \cdot d_k) \quad (1)$$

where DBH_k is the diameter at breast height of tree k, c is a scaling factor, and d_k is the distance from point p to tree k. For simplicity the scaling factor c was set to 1. Using an exponentially decreasing function, the influence of a tree was considered to be negligible at distances beyond 5 m ($r' = 5$). When the combined influence of trees is described with this index, it is assumed that (1) the influence of a tree is strongest near the stem and decreases with distance from the tree, (2) the influence of a tree is symmetrical in space, (3) the influence is proportional to the size of the tree as described by the diameter at breast height (DBH), and (4) the effects of trees are additive.

The last assumption makes it possible to use multiple linear regression to relate mole percentage and PC-scores from the 2 m grid to the calculated IPs for spruce and birch, respectively, and to estimate a regression coefficient of influence (CoI) for each tree species. Variograms revealed that observations of many variables were spatially autocorrelated at a distance of 2 m, and thus P-values from the regression, analysis are misleading. To correct for autocorrelations in the regression, analysis is analytically complicated. We instead chose a conservative value of $P = 0.001$ as the lowest significance level.

Variables with a positively skewed distribution were log-transformed prior to the statistical analysis to normalise probability distributions (Webster and Oliver, 1990).

3. Results

The total amount of PLFAs fell within the range of 1.0–2.0 μmol g^{-1} organic matter and the coefficient of variation (CV) was 0.15 (data not shown). The proportion of bacterial PLFAs varied between 0.38 and 0.47, with a CV of 0.11 (Table 1). Single PLFAs (expressed as mol percentage) generally had a CV between 0.13 and 0.28, except for PLFAs which were found in low concentrations. These PLFAs (e.g., 15:0, 19:1b and 19:1a) were less precisely quantified by the method, and had a CV between 0.33 and 0.46.

The 32 most common PLFAs (expressed as mol percentages) were subjected to a principal components analysis (PCA, Fig. 2), where the first two principal components accounted for almost 50% of the variation in the PLFA pattern. The first component was characterised by relatively low proportions of 16:1ω5, 16:1ω7c, 16:1ω9, and 18:1ω7, and relatively high pro-

Table 1
Coefficient of variation and variogram model parameters for spatially structured PLFAs. The range is the limit of spatial dependence. Spatial structure is the fraction of spatially related semivariance (C) to total semivariance ($C+C_0$). PLFAs in Fig. 2 not included here were not clearly spatially structured. Model variograms were fitted to the experimental semivariances with the exponential model for 18:1ω7 and with the spherical model for all other PLFAs

PLFA	CV	Range (m)	Spatial structure	Model fit r^2
i16:0	0.18	1.0	0.61	0.89
10Me17:0	0.19	1.2	0.41	0.97
br18:0	0.26	1.3	0.77	0.89
10Me16:0	0.16	1.4	0.57	0.98
cy19:0	0.16	1.5	0.68	0.98
16:1ω5	0.28	1.6	0.76	0.96
18:2ω6,9	0.34	2.0	0.68	0.92
16:0	0.15	2.1	0.93	0.96
br17:0	0.18	2.3	0.64	0.96
i16:1	0.22	2.4	0.51	0.97
16:1ω7c	0.24	2.5	0.66	0.98
17:0	0.15	2.5	0.84	0.99
i17:0	0.17	3.8	0.75	0.96
17:1ω8	0.20	3.8	0.45	0.89
a17:0	0.16	4.6	0.89	0.98
18:1ω9	0.20	5.0	0.46	0.94
19:1b	0.37	5.1	0.85	0.96
18:1ω7	0.25	5.3	0.66	0.96
16:1ω7t	0.18	6.9	0.59	0.94
10Me18:0	0.21	11.3	0.59	0.99
Bacterial PLFAs	0.11	4.9	0.63	0.96

portions of x5, 19:1b, and 20:0; while the second component was characterised by high amounts of 16:1ω5, 16:1ω7c, 18:1ω9, and 18:2ω6,9, and relatively low amounts of several branched PLFAs (10Me16:0, 10Me17:0, i16:0, i16:1, br17:0, and br18:0).

Twenty of the 32 examined PLFAs were spatially autocorrelated. The experimental variogram for the other 12 PLFAs could not be separated from pure nugget variation due to poor fit of the model variogram or a high nugget to sill ratio. The limit of spatial dependence (the range) varied between 1 m and 11 m, and the proportion of sill that was spatially structured ranged from 0.41 to 0.93 (Table 1). The PLFAs that showed the shortest range of spatial autocorrelation, (e.g., i16:0, 10Me17:0, br18:0, and 10Me16:0) were all members of the cluster with large negative loadings along the second principal component (Fig. 2). The proportion of bacterial PLFAs had a range of approximately 4.9 m. The variation of the microbial community structure, as characterised by the PLFA pattern, showed strong spatial continuity at the scale studied, as indicated by the high proportion of each sill showing spatial structure (Fig. 3). The first two principal components described variation structured at two different spatial scales. The range of the microbial community for the first component was 4.6 m, whereas that for PC 2 was only 1.5 m (see also Fig. 4).

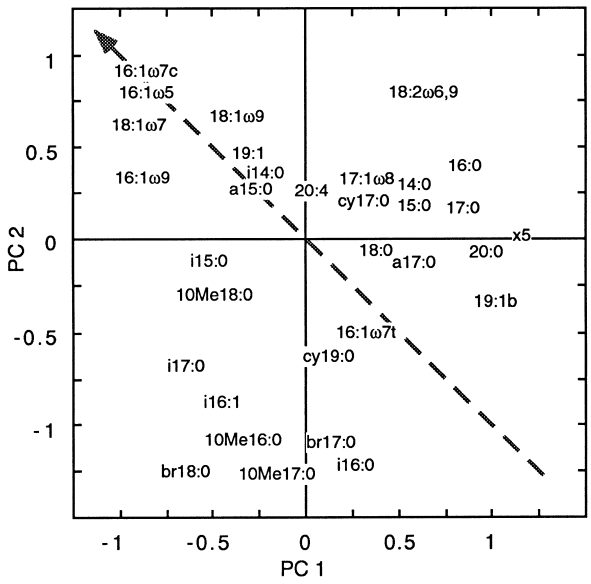

Fig. 2. PCA plot for soil microbial community structure (phospholipid fatty acid, PLFA) pattern, showing loadings of single PLFAs along the first two principal components. The first axis was positively correlated to the IP_{spruce} and explained 32% of the variation in PLFAs. The second axis was positively correlated to the IP_{birch} and explained an additional 16% of the variation in PLFAs. The diagonal dashed arrow indicates the combined spruce–birch influence, with high spruce and low birch influence at the lower right corner of the plot and vice versa. PLFA scores projected onto this axis were used to compare the results from this study with those from a laboratory study (see Fig. 5 and Section 4).

The microbial community appeared to be influenced by the position of the trees (Table 2, Fig. 4). The first principal component was related to spruce ($r^2 = 0.41$), whereas the second principal component was more weakly related to birch ($r^2 = 0.14$). Thus, sample scores along the first component increased with spruce influence potential, and scores along the second principal component increased with birch influence potential. The influence of the trees was also seen in some of the individual PLFAs, since 12 of the PLFAs were influenced by the calculated tree influence potentials (Table 2). All of these PLFAs were influenced by spruce trees; seven increased with spruce influence and five decreased. Only one PLFA (16:1ω7c) was also significantly influenced by birch trees. The proportion of bacterial PLFAs decreased with spruce influence potential but was unaffected by birch influence potential.

4. Discussion

The composition of the microbial community appeared to be spatially structured at the scale studied, i.e. between 0.2 and 20 m. The majority of the PLFAs studied showed variation at scales one order of magnitude greater than the fatty acids in an agricultural field (Cavigelli et al., 1995). Furthermore, the set of fatty acids that were spatially autocorrelated in the present study (Table 1) were not among those that exhibited variation on the scale of soil aggregates and rhizo-

Table 2
Influence of Norway spruce and birch on single PLFAs, bacterial PLFAs and the PLFA pattern (first two principal components, PC 1 and PC 2, see Fig. 2). The coefficients of influence (CoI) were derived from the regression $Y = aIP_{spruce} + bIP_{birch}$ ($n = 96$); see Section 2. The dependent variable was normalised prior to the regression to make regression coefficients directly comparable

Variable (Y)	Spruce CoI (a)	Birch CoI (b)	Full model (r^2)
x5	1.20[a]	0.16	0.24
19:1b	1.17[a]	−0.24	0.28
17:1ω8	1.08[a]	0.40	0.18
17:0	1.00[a]	−0.34	0.22
14:0	0.97[a]	0.37	0.14
15:0	0.84[a]	0.43	0.10
a17:0	0.83[a]	−0.43	0.17
16:1ω7c	−0.72[a]	0.71[a]	0.20
16:1ω5	−1.08[a]	0.53	0.30
i17:0	−1.17[a]	−0.48	0.21
18:1ω7	−1.20[a]	0.33	0.31
16:1ω9	−1.33[a]	−0.27	0.29
Bacterial PLFAs	−0.95[a]	0.11	0.16
PC 1 scores	1.51[a]	−0.01	0.41
PC 2 scores	−0.26	0.92[a]	0.14

[a] Statistically significant coefficients.

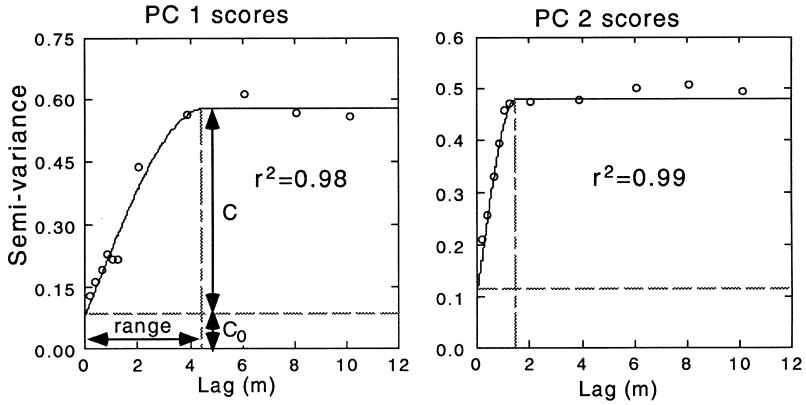

Fig. 3. Variograms of soil microbial community structure (PLFA pattern) in a mixed Norway spruce–birch stand. Variograms were constructed using scores along the first and second principal components from PCA of PLFA patterns, using the spherical model.

sphere (<0.2 m) in the agricultural field (e.g., i14:0, 15:0, a15:0 and cy17:0). Thus, we suggest that the more widely spaced and long-lived trees in this study induced spatial variation on a larger scale than annual row crops on an agricultural field, and that the microbial communities in the stand studied may have been different from that in an arable soil. However, the use of different lipid fractions, phospholipids in our study and total lipids in the study by Cavigelli et al. (1995), of course can not be ruled out as a possible explanation for the different results.

We have demonstrated that individual PLFAs in a forest may vary at scales from 1 m to 11 m, but we have no detailed information about the processes that may have induced patterns on these scales. However, from the analysis of how PLFAs were related to the influence potentials of trees, it is clear that Norway spruce trees influenced the soil microbial community to a larger extent than did birch trees. Spruce trees appeared to induce spatial patterns in 12 PLFAs, total bacterial PLFAs, and the microbial community structure along the first principal component. The patterns induced by spruce trees had a characteristic patch size of 4–5 m. That is, the median range of PLFAs influenced by spruce was 3.8 m; the total bacterial PLFAs, which decreased with spruce influence, had a range of

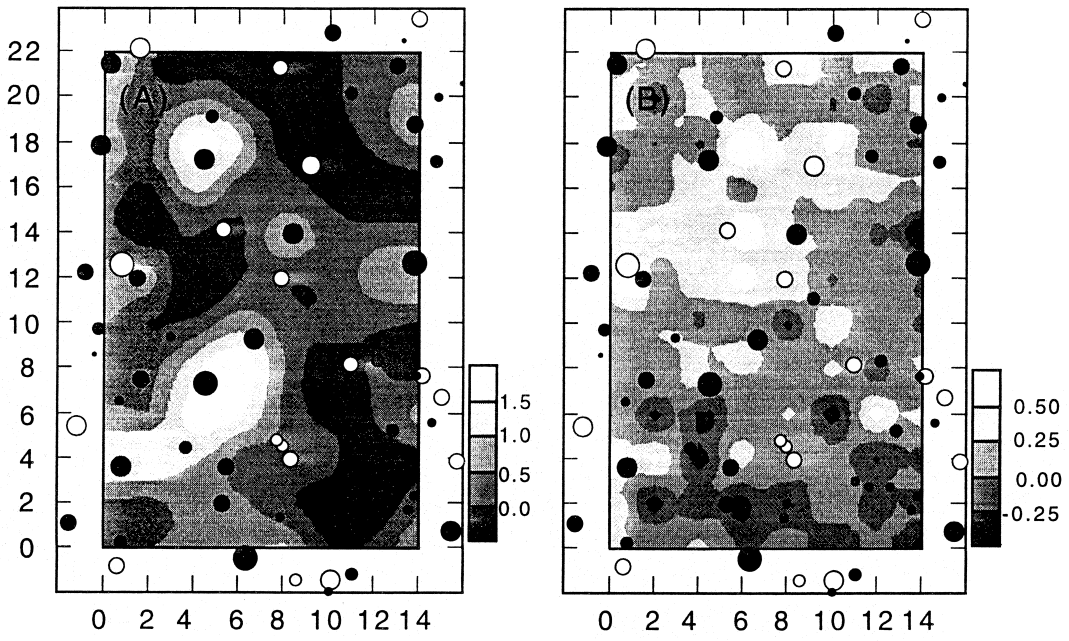

Fig. 4. Spatial patterns of the soil microbial community structure, as characterised by the PLFA pattern, in a mixed spruce–birch stand: (a) scores along the first principal component. (b) scores along the second principal component. For PLFAs loading along the principal components see Fig. 2. Grey and white circles represent spruce and birch trees respectively. The size of the circle is proportional to the diameter of the tree at breast height. Spatial scale is in metres.

4.9 m (Tables 1 and 2); and the microbial community structure described by the first principal component formed patches with a characteristic size of 4.6 m (Figs. 3 and 4(a)).

It is not self-evident that the influence of the trees on the PLFA pattern should be assumed to be largest near the stem (Eq. 1), since root mass and metabolic activity can be located away from the tree. However the significant correlations between PLFAs and the calculated influence potentials indicate that this is the case (Table 2). Changes in the microbial community associated with spruce may partly reflect a response to the patchy forest floor environment created by spruce trees in this stand. Both light radiation (PAR) reaching the ground and water content of soil decreased with spruce influence potential, as did the cover of *Brachytecium reflexum* and associated herb species (Saetre, 1999). It is also possible that the properties of the organic matter were influenced by the spruce trees and that this in turn influenced the microbial community structure. The influence of trees on soil chemical properties may decrease with the distance from stems (Falkengren-Grerup, 1989; Koch and Matzner, 1993), and the soil closest to the trunk has presumably a longer history of influence from the canopy and the roots than soil away from the tree trunks.

The influence of spruce and birch on PLFAs and the PLFA pattern found in this study can be compared to the changes in PLFA pattern along a laboratory spruce–birch soil gradient (Saetre, 1998). In the experiment, soil from a pure Norway spruce and a pure birch (*Betula pendula*) stand were used to prepare microcosms in a soil replacement series, from pure spruce soil to pure birch soil, with the soil mixtures 3:1, 1:1 and 1:3 in between. The influence of the spruce and birch trees on the composition of PLFAs appeared to be similar in the laboratory study and in this study. For example, of the 12 PLFAs that were significantly influenced by trees in this study (Table 2), eight PLFAs responded in the same way to spruce influence potential in the field and to spruce soil content in the microcosm experiment.

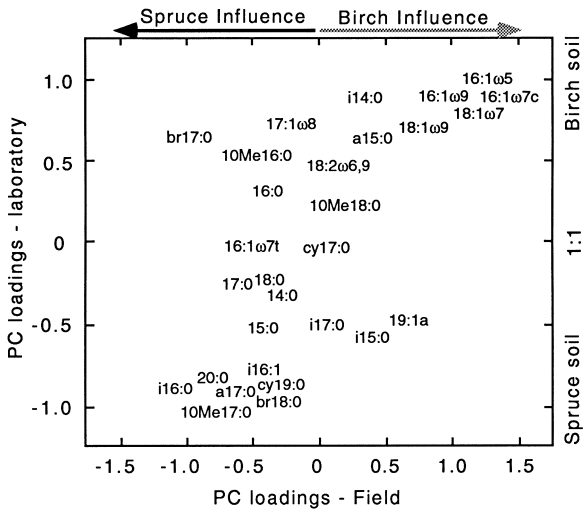

Fig. 5. A comparison of the influence of spruce and birch trees on microbial community structure (PLFA pattern) in a mixed forest with that reported from a microcosm study (Saetre, 1998). The horizontal axis indicates the combined spruce–birch influence on the PLFA pattern in the field study: PLFAs to the left were found in high proportions in areas with high spruce or low birch influence potential and vice versa. The vertical axis reflects the change in PLFA pattern along a laboratory spruce–birch soil replacement series: the relative amounts of PLFAs with negative scores along this axis decreased with increasing proportion of birch soil, whereas the amounts of PLFAs with positive scores increased with the proportion of birch soil.

A more formal comparison of the responses of the microbial community to tree influence in the two studies can be made by comparing the PLFA loadings along principal components from the two studies. Such a comparison shows that changes in the microbial community that were associated with trees in this field study were consistent with the PLFA patterns along the laboratory spruce–birch soil gradient ($r = 0.62$, $P < 0.001$), although a few PLFAs (e.g., br17:0, i15:0 and 19:1a) deviated from the general pattern (Fig. 5). The vertical axis in Fig. 5 reflects individual PLFA loadings found in the laboratory spruce–birch soil gradient described above (Saetre, 1998): the concentration of PLFAs with negative loadings decreased with the proportion of birch soil, whereas the concentration of PLFAs with positive loadings increased with birch soil content. This means that positive loadings along this component either are the result of PLFAs being positively associated with birch soil or of PLFAs being negatively associated with spruce soil. In the present field study the influence of birch and spruce was not found in one single principal component, but instead the spruce influence increased with positive loadings along PC 1 and the birch influence with positive loadings along PC 2. Thus, the PLFA scores along the first two principal components in this study were projected on to one diagonal axis (dashed arrow in Fig. 2; horizontal axis in Fig. 5), in order to make the PLFA loadings comparable between the field study and the laboratory experiment. Along this new axis, PLFAs that were positively associated with spruce influence (PC 1) and PLFAs that were negatively associated with birch influence (PC 2) had negative loadings, whereas PLFAs that were either positively influenced by birch trees or negatively influenced by spruce trees had positive loadings. Thus, PLFAs 16:1ω7c, 16:1ω5, 18:1ω7, and 18:1ω9 increased with the proportion of birch soil in the laboratory experiment. In the field these PLFAs increased with birch influence but decreased with spruce influence. Furthermore, PLFAs 20:0, a17:0, 10Me17:0, and br18:0 increased with the

proportion of spruce soil in the experiment and were either positively influenced by spruce (20:0, a17:0) or negatively influenced by birch (10Me17:0, br18:0) in the field.

These similarities between the field and laboratory studies suggest that the influence of tree species on the microbial community is partly through their influence on the quality of organic matter. That is, although spruce and birch trees differed in their influence on, for example, soil moisture and ground vegetation, the observed differences in the field between species, with respect to how they influenced the microbial community, remained in soil incubated for 3 months under laboratory conditions (with no differences in soil moisture and vegetation). Differences in both above and below ground litter between spruce and birch may have contributed to differences in organic matter quality. Soil pH does not appear to be a factor behind the similar results from the field and laboratory studies, since pH varied only marginally in the field study (CV = 0.02) and pH was not related to the position of trees in the study area (Saetre, 1999).

There were only two PLFAs that were spatially dependent on distances clearly greater than 5 m, but quite a few that had a range of 2 m or less. It is interesting to note that among the PLFAs that showed small scale spatial patterns many were correlated to each other and loaded heavily on the second principal component. For example, i16:0, i16:1, 10Me16:0, 10Me17:0, br17:0, and br18:0 formed a cluster in the principal components analysis and had an average range of 1.6 m (Fig. 2, Table 2). All these branched PLFAs are considered to be typical for Gram-positive bacteria (O'Leary and Wilkinson, 1988), and the cluster of branched PLFAs might thus represent a complex of strongly associated organisms that aggregated in small patches. Their negative loadings on the second principal component implies that patches with a high proportions of these PLFAs were found away from birch trees (see dark patches with low PC 2 scores in Fig. 4(b)). The fact that patches were found at distances from birch trees that exceeds the limit of spatial dependence for the second principal component (1.5 m), seems to imply that the relatively fine grained patchiness of these organisms was unrelated to birch trees, but the location of patches was to some extent influenced by the position of the birch trees.

The soil under birch trees had been shown to be associated with relatively high soil respiration rates as compared to soil away from trees or soil under spruce trees (Saetre, 1999), and in the laboratory experiment with the spruce–birch soil replacement series, birch soil appeared to have a more active microflora (Saetre, 1998). Increased activity of the soil microorganisms is also found after liming or application of wood ash to forest soils (e.g., Persson et al., 1989; Pietikainen and Fritze, 1993; Bååth and Arnebrant, 1994). These treatments resulted in increased amounts of the PLFAs 16:1ω5 and 16:1ω9, and to a less extent of 16:1ω7c and 18:1ω7 (Frostegård et al., 1993a, 1993b; Bååth et al., 1995). These four PLFAs were the ones that increased most with the birch influence potential and birch soil content (Fig. 5), thus suggesting that these PLFAs might be indicative of especially active microorganisms in these forest soils.

We conclude that the spatial patterns of PLFAs in a forest may be on a scale one order of magnitude higher than that found in an agricultural field, and that patterns in the microbial community are associated with the position of trees. Norway spruce trees had a much stronger influence on PLFA patterns than birch trees, and spruce trees seemed to induce patches at a scale of 4–5 m in the studied stand. A comparison with a laboratory experiment suggests that although the tree species differ in their influence on, for example, soil moisture and ground vegetation, their influence on the microbial community were to a large extent connected to differences in the quality of soil organic matter associated with the two tree species.

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