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Effects of mesofauna in a spruce forest on soil microbial communities and N cycling in field mesocosms

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Abstract

Using field mesocosms maintained for 6 months in an acid spruce forest, we investigated the influence of microarthropods on biomass, structure and function of the soil microbial community. In the litter layer (L/F layer), the re-immigration of mesofauna into mesocosms did not significantly affect substrate-induced respiration (SIR), biomass C, biomass N, biomass P, N-mineralisation or enzymes involved in N cycling. There was no effect of the mesofauna on the biomarkers for fungal biomass (ergosterol and phospholipid fatty acid 18:2ω6) in the litter layer. Mesofauna activities increased microbial biomass (biomass C, N and P) in the H layer, and significantly increased soil protease activity and phosphate content in the H layer. Since biomass P did not change significantly when mesofauna recolonised mesocosms, they presumably affected P-mineralisation by producing P-rich faeces. Discriminant analysis showed that mesofauna affected microbial N-mobilisation in the H layer. Higher protease and arginine deaminase activities in mesocosms with mesofauna may have been caused by faunal grazing on soil microorganisms.

Keywords: Spruce forest; Mesofauna; Soil microbial communities; N transformations; Mesocosm microbial–faunal interactions

1. Introduction

Soil mesofauna affect the structure and activity of microbial communities and enhance nutrient turnover (Coleman, 1986; Verhoef and Brussaard, 1990; Lussenhop, 1992). The mechanisms of the effects have been demonstrated in a number of studies. Substrate comminution and mixing by microarthropods were associated with increased metabolic rates of bacteria (Lussenhop, 1992). Fungal grazing by microarthropods was accompanied by a changing growth pattern and by the release of enzymes of fungi (Bengtsson et al., 1993). The grazing activity of enchytraeids modified the metabolic activities of microbial communities (Förster et al., 1995). Beside mesofauna-induced changes in the activities of soil microorganisms, selective grazing may alter the competitive relationships between fungi (Newell, 1984) and may increase the rate at which primary saprophytes are replaced by secondary saprophytes (Klironomos et al. 1992). In contrast, in other studies, grazing by *Oppiella nova* and *Onchychiurus subtenuis* did not affect fungal species richness, diversity, dominance, or frequency of occurrence of 29 fungal taxa (including taxa known to be preferred or avoided by the animals) (McLean et al., 1996).

The microbial–faunal interaction can be modified by several external factors. Collembolans influence the nutrient availability of mycorrhiza (Lussenhop, 1996), while temperature, moisture and environmental chemi-

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cals may have synergistic or antagonistic influences on the interactions between microorganisms and mesofauna (Förster et al., 1995). Therefore, there is a need for studies of microbial faunal interactions under field conditions. Field enclosures (Verhoef and de Goede, 1985; Faber and Verhoef, 1991) and mesocosms (Bruckner et al., 1995; Kampichler and Kandeler, 1996) have been used to quantify the effects of microbial–faunal interactions in the field. Whereas these studies showed the influence of mesofauna on activities of soil microorganisms, much less information is available about the influence of soil fauna on the structure of natural microbial communities.

Our aim was to determine whether the soil mesofauna affects the biomass, structure and function of the soil microbial community in an acid spruce forest under field conditions in Austria. The microbial community was characterised by its carbon, nitrogen and phosphorus content and by its total content of phospholipid fatty acids (Tunlid and White, 1992). We attempted to link these indices of biomass and community structure of soil microorganisms to the functioning of soil microorganisms. The combined estimation of nutrient release, N-mineralisation and enzymes involved in nitrogen cycling was expected to help the assessment of the role of mesofauna in N cycling: the predominance of a direct influence on N cycling through faeces and excreta would be recognized by the enhancement of N mobilisation without a significant increase in microbial activity, whereas a stimulated microbial activity would characterise the significance of an indirect influence.

2. Materials and methods

2.1. Study site

The mesocosm experiment was carried out in a 40-year old *Picea abies* forest with no understory located in the Gleinalm region near Knittelfeld in Styria, Austria ('Stangelwald' forest, 47°13′N, 14°59′E, National Grid Reference BMN 6705-4830-1b, 1040 m above sea level, mean annual temperature 6.2°C at 700 m above sea level, 2.8°C at 1600 m; annual precipitation 600–850 mm). The soil was classified as a loamy sandy Dystric Cambisol (FAO classification) with a mor humus layer (L, F and H layers, thickness up to 6 cm). Organic carbon and total N in the L/F horizon ranged from 394–448 mg g^{-1} and from 17.1–18.3 mg g^{-1}, respectively; the pH (10 mM $CaCl_2$) ranged from 3.2–3.9.

2.2. Design of the mesocosm experiment

Ten monoliths (25 × 25 × 20 cm) were taken randomly in April 1995. After defaunation of the monoliths for 10–12 h at −40°C with solid CO_2, the monoliths were wrapped in nets of various mesh size and randomly replaced at the study site (Bruckner et al., 1995). Soil mesofauna was introduced into five of the mesocosms using a field Berlese-Tullgren device to establish an assemblage of microarthropods mimicking natural conditions. Approximately 3 l of H and L/F humus material (randomly taken at the study site and slightly mixed) were placed in each field Berlese-Tullgren device and processed in successive runs. Microarthropods were forced into the mesocosms by heating with a plastic plate (integrated heating wires) which directly rested upon the humus material. The heating plates were connected to the power supply system via two 24 V-transformers. The temperature of the humus material was adjusted and automatically levelled with an electronic feedback mechanism (raised from 25 to 35°C during extraction). After 4 d, the humus material was dry and the extraction terminated. The re-introduction of the microarthropods into the mesocosms was completed after an extraction period of 4 d. We prepared the following treatments:

1. Microbiota: five defaunated monoliths were wrapped in fine nets (35 μm mesh size). This treatment was designed to exclude the influence of mesofauna, macrofauna and roots.
2. Microbiota and mesofauna: five defaunated monoliths were wrapped in coarse nets (1 mm mesh size) and inoculated with microarthropods. The coarse nets were designed to allow the lateral re-immigration of mesofauna during the 6 months incubation. Therefore, this treatment tested effects of mesofauna on microorganisms.
3. Controls: ten randomly selected areas (25 × 25 cm) were chosen as control plots and left intact until sampling. The control is also referred to as treatment 3 ('microbiota + complex fauna + living roots').

The comparison of the two types of mesocosms and the controls allowed us to evaluate the effects of different abundance of fauna in the mesocosms as well as possible side effects of the manipulation such as deep-freezing and cutting of living roots.

The mesocosms were destructively sampled after 6 months of exposure. To determine the efficiency of the introduction of microarthropods into treatment 2 mesocosms (microbiota and mesofauna) by the field Berlese-Tullgren equipment and the efficiency of exclusion of microarthropods from treatment 1 mesocosms (microbiota), two soil cores (dia 7 cm, 10 cm depth) were taken from each mesocosm and control. These cores were extracted in a Berlese-Tullgren device for 1 week (Kampichler et al., 1999). Enchytraeids were not monitored since they are known to be efficiently elimi-

nated and excluded from treatment 1 mesocosms and efficiently immigrate into defauneated treatment 2 mesocosms and attain field density (Bruckner et al., 1995).

The remaining mesocosms were separated into litter (L and F) and humus layer and used for microbiological analyses. Field moist samples were stored in plastic bags at −20°C. After the storage period (up to 2 months), samples were allowed to thaw at 4°C for about 3 d. The samples were sieved (< 5 mm) and stored in plastic bags at 4°C; microbial analyses started within 2 weeks.

2.3. Soil chemical analyses

Soil pH was measured in 10 mM $CaCl_2$ solution (soil to solution ratio 1:12.5). NH_4^+ and NO_3^- was extracted in a $CaCl_2$ solution (12.5 mM); NH_4^+ was analyzed colorimetrically by a modified indophenol reaction (Kandeler and Gerber, 1988) and NO_3^- was measured colorimetrically as NO_2^- after reduction using a Cd-column (Morris and Riley, 1963). The fraction of plant-available P was measured colorimetrically after extraction of P with a calcium lactate solution (19 mM, pH 3.6; soil to solution 1:50) according to Hoffmann and Ohnesorge (1966). TOM (total organic matter) and total N was determined after dry oxidation of organic matter in an O_2 stream with an automated CNS analyser (LECO) (Schinner et al., 1996).

2.4. Soil microbial analysis

2.4.1. Microbial biomass

For the substrate-induced respiration (SIR) measurement, substrate saturation and maximum initial respiration response were obtained with an amendment rate of 8.0 mg glucose g^{-1}. CO_2 evolved was trapped in 50 mM NaOH for a 4 h incubation at 25°C and measured by titration (Anderson and Domsch, 1978, modified by Jäggi, 1976).

Soil microbial biomass C was measured by fumigation extraction (Vance et al., 1987) as modified by Joergensen (1995a, b). Moist soil was split into two portions (5 g for the fumigated and 5 g for the nonfumigated treatment), extracted on a horizontal shaker at 200 rpm with 100 ml 10 mM $CaCl_2$ and filtered through a folded paper filter (Schleicher and Schuell 595 1/2). Organic C in the extracts was measured as CO_2 after catalytic combustion at 800°C using a Maihak Tocor 2 automatic analyser. Soil microbial biomass was estimated from the relationship: biomass $C = E_C/k_{EC}$, where E_C is (organic C extraced from fumigated soil) minus (organic C extracted from nonfumigated soil) and $k_{EC} = 0.45$ (Joergensen, 1995a, b).

For the estimation of biomass nitrogen (N_{mic}), litter and soil samples were fumigated with chloroform for 24 h. Ninhydrin-reactive N was determined after extraction with 2 M KCl using the colorimetric procedure according to Amato and Ladd (1988) as modified by Joergensen and Brookes (1990). N_{mic} (μg N_{mic} g^{-1} soil) was calculated as (mg ninhydrin-reactive N g^{-1} soil × 3.1(according to Amato and Ladd (1988).

Soil microbial P was also measured by fumigation extraction (Brookes et al., 1982). The moist soil was split into three portions equivalent to 1 g dry weight. The first aliquot (nonfumigated control) was placed in a 250 ml centrifuge bottle and then extracted with 100 ml of 0.5 M $NaHCO_3$ (pH 8.5) for 30 min by oscillating shaking at 150 rev min^{-1}. The resulting suspension was centrifuged (2000*g*) and then filtered through a paper filter (Schleicher and Schuell 595 1/2). The second portion (nonfumigated recovery control) was also placed in a 250 ml bottle and then extracted as described above with 100 ml of 0.5 M $NaHCO_3$ and additionally 1 ml of KH_2PO_4 solution containing 250 μg P as a spike equivalent to 25 μg P g^{-1} soil. The third portion was fumigated for 24 h at 25°C. After removal of $CHCl_3$ by repeated evacuation, extraction was performed as for the nonfumigated control. The extract was filtered (Schleicher and Schuell, 595 1/2) a second time immediately before P was analysed by a modified ammonium molybdate-ascorbic acid method as described by Joergensen (1995b). Microbial biomass *P* was calculated as follows: microbial biomass $P = E_P/k_{EP}$, where $E_P = [(F-U)/(S-U)]$, F = (extractable PO_4–P in fumigated soils), U = (extractable PO_4–P in nonfumigated soils), S = (extractable PO_4–P in nonfumigated soils + spike of 25 μg P g^{-1} soil) and $k_{EP} = 0.40$ (Brookes et al., 1982).

2.4.2. Ergosterol

Moist soil (2 g) was extracted with 100 ml ethanol for 30 min by oscillating shaking (250 rpm). The soil suspension was filtered (Whatman GF/A) immediately after extraction, the extract being evaporated in a vacuum rotary evaporator at 40°C in the dark. The residue was collected in 10 ml ethanol and filtered again (cellulose acetate membrane 0.45 μm). Quantitative determination of ergosterol was performed by reversed-phase HPLC analysis at 25°C by using a main column of 12.5 cm Spherisorb ODS II S% (Knauer Vertex 12 cm main-column, 0.5 cm pre-column) with a mobile phase of 97% methanol/3% water (v/w), a flow of 1.1 ml min^{-1} and resolution of detection of 282 nm. Retention of ergosterol with this system is 7.25 ± 0.25 min (Djajakirana et al., 1996).

2.4.3. Phospholipid fatty acids (PLFAs)

Lipids were extracted from litter samples using a one-phase extraction procedure and fatty acids were separated using SPE columns according to the method described by Zelles and Bay (1993). Briefly, 25 g of

Table 1
Abundance of Acarina and Collembola (10^3 individuals m^{-2}) in field mesocosms and undisturbed soil (control) after exposure for 6 months in a spruce forest. Means of the treatments followed by the same letter are not significantly different at $P < 0.05$ (Tukey's highest significant difference)

	Mesocosm including		Control
	microbiota	microbiota and mesofauna	microbiota, complex fauna and roots
Acarina	26.06 a	111.58 b	383.41 c
Collembola	17.35 a	171.95 b	129.62 ab

moist soil were extracted with a one-phase mixture consisting of chloroform (125 ml), methanol (250 ml) and phosphate buffer (90 ml, 50 mM K_2HPO_4; pH 7.4) for 2 h. The suspension was divided into two phases by adding 125 ml chloroform and 125 ml distilled water and by allowing them to separate for 24 h. The lower phase was collected and reduced to a volume of 10 ml using a rotary evaporator. One ml of this lipid extract was fractionated into neutral, glyco- and phospholipids on an SI-solid phase extraction (SPE) column. The phospholipid fraction was subjected to a mild alkaline methanolysis to obtain fatty acid methyl esters (FAMEs). Then the FAMEs were separated into unsubstituted and hydroxy-substituted fatty acids (PLOHs) using an NH_2 SPE-column. The unsubstituted FAMEs were further separated into saturated (SATFAs), monounsaturated (MUFAs) and polyunsaturated (PUFAs) subgroups by silver ion chromatography. Derivations were necessary for MUFAs and PLOHs. All FAMEs and their derivates were analysed with GC-MS (Hewlett-Packard 5971 A MDS combined with 5890 series II GC system). Fatty acid nomenclature was used as described by Frostegård and Bååth (1996). The concentrations of individual fatty acids were expressed as the portion (mol%) of the sum of all fatty acids. The content of the fatty acid 18:2ω6 was used as an indicator of fungal biomass. The sum of 12 different fatty acids (i15:0, a15:0, n15:0, i16:0, 16:1ω916:1ω7, i17:0, a17:0, n17:0, cy17:0, 18:1ω7 and cy19:0) was used to represent bacterial PLFAs (bactPLFAs) (Frostegård and Bååth, 1996).

2.4.4. Soil microbial processes

N-mineralisation was measured by incubating soil samples (5.0 g) under waterlogged conditions in an enclosed tube at 40°C for 7 d; the production of ammonium was measured by a colorimetric procedure (Kandeler and Gerber, 1988). Protease activity was determined using the method of Ladd and Butler (1972). The samples were incubated for 2 h in a buffered casein solution (pH 8.1) at 50°C. The aromatic amino acids released were extracted with trichloroacetic acid (0.92 M) and measured colorimetrically after addition of the Folin-Ciocalteu reagent. The method used for estimating urease activity involves incubation of the soil with an aqueous urea solution (for 2 h at 37°C), extraction of ammonium with 1 M KCl and 10 mM HCl, and colorimetric NH_4^+ determination by a modified indophenol reaction (Kandeler and Gerber, 1988). Soil deaminase activity was measured colorimetrically by deamination of an aqueous arginine solution (incubation for 2 h at 37°C), as described by Alef and Kleiner (1986).

2.4.5. Analytical methods and statistical procedure

All analytical results were calculated on the basis of oven-dry (105°C) weight of soil. Microbial biomass and soil microbial processes were determined in duplicate. Variables of soil microbial properties were tested for normality (Kolmogorov–Smirnov) and homogeneity of variances (Cochran's C). If necessary, data were log-transformed prior to the analysis. Differences between means were inspected by a one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference test.

Biomass-related variables (SIR, C_{mic}, N_{mic}, P_{mic}, ergosterol) and variables related to the cycling of N (N-mineralisation, protease activity, deaminase activity and urease activity) were analysed by discriminant analyses for each layer. UPGMA cluster analysis using the Ochiai coefficient (cosine) as a similarity index was applied for log-transformed data of PLFA concentrations according to Jongman et al. (1996). By this procedure we tested whether the clusters defined by the similarity between single objects (mesocosms) corresponded with the groups defined by the three treatments.

3. Results

3.1. Abundance of mesofauna

In treatment 1 mesocosms, the mean densities of collembolans and mites were only 17,000 and 26,000 individuals m^{-2}, respectively. Thus, the total field density of microarthropods (500,000 individuals m^{-2}) was reduced by 91.5% (Table 1). Collembola density in treatment 2 was higher than in the control. In contrast,

Table 2
Chemical and microbiological properties in the L/F horizon in field mesocosms and undisturbed soil (control) after exposure for 6 months in a spruce forest. Means of the different treatments followed by the same letter are not significantly different at $P < 0.05$ (Tukey's highest significant difference)

Soil properties	Mesocosm including		Control	*F*	*P*
	microbiota	microbiota and mesofauna	microbiota, complex fauna, roots		
Water content (%)	65.6 a	66.3 a	52.8 b	32.278	***
C_{org} (mg g^{-1})	394	402	448	2.6799	n.s.
N_{org} (mg g^{-1})	17.3 ab	17.1 a	18.3 b	5.116	*
C-to-N ratio	22.7	23.5	24.4	0.9640	n.s.
pH ($CaCl_2$)	3.56 ab	3.71 a	3.35 b	10.308	**
NH_4^+ (μg N g^{-1})	188.9 a	188.4 a	115.6 b	5.691	*
PO_4^{3-}(μg P g^{-1})	23.02	26.62	28.37	0.816	n.s.
SIR (mg CO_2 100 g^{-1} h^{-1})	36.15	36.76	39.46	0.238	n.s.
Biomass C (μg C g^{-1})	5217 a	5314 a	6829 b	7.651	**
Biomass N (μg N g^{-1})	505	499	431	0.551	n.s.
Biomass P (μg P g^{-1})	261	248	255	0.092	n.s.
Ergosterol (μg ergosterol g^{-1})	156.7 ab	125.9 a	190.1 b	5.045	*
EL-$PLFA_{total}$ (nmol g^{-1})	1500	1137	1108	2.455	n.s.
PLFAs of fungi (mol%)	5.24	5.08	6.30	1.192	n.s.
PLFAs of bacteria (mol%)	29.43	30.65	28.94	1.919	n.s.
N-mineralisation (μg N g^{-1} 7 d^{-1})	234.1	211.6	269.1	1.141	n.s.
Protease (μg tyrosine g^{-1} 2 h^{-1})	6530	7286	5074	3.082	n.s.
Arginine deaminase (μg N g^{-1} 2 h^{-1})	64.4	66.3	48.1	2.311	n.s.
Urease (μg N g^{-1} 2 h^{-1})	703.7	715.8	671.4	0.162	n.s.

mites did not attain the abundance of the undisturbed field situation. However, since total microarthropod density in treatment 2 was 6.5× that in treatment 1, comparing the treatments allows us to judge the effects of the mesofauna.

3.2. Chemical properties

The water content of L/F and H horizon of the mesocosms was significantly higher than that of the corresponding layers of the control (Tables 2 and 3).

Table 3
Chemical and microbiological properties in the H horizon in field mesocosms and undisturbed soil (control). Means of the different treatments followed by the same letter are not significantly different at $P < 0.05$ (Tukey's highest significant difference)

Soil properties	Mesocosm including		Control	*F*	*P*
	microbiota	microbiota and mesofauna	microbiota, complex fauna, roots		
Water content (%)	56.5 ab	61.6 a	49.4 b	6.112	*
C_{org} (mg g^{-1})	241	281	276	0.880	n.s.
N_{org} (mg g^{-1})	12.9	14.7	14.8	1.512	n.s.
C-to-N ratio	18.5	19.0	18.7	0.121	n.s.
pH ($CaCl_2$)	3.43 a	3.47 a	3.24 b	9.199	**
NH_4^+ (μg N g^{-1})	123.9	182.8	129.4	4.726	n.s.
PO_4^{3-} (μg P g^{-1})	4.73	12.53 b	8.72 a	10.131	**
SIR (mg CO_2 100 g^{-1} h^{-1})	15.7	20.6	17.4	1.003	n.s.
Biomass C (μg C g^{-1})	3144	3640	3764	2.296	n.s.
Biomass N (μg N g^{-1})	211 ab	262 a	201 b	5.529	*
Biomass P (μg P g^{-1})	126.8	164.5	138.8	2.441	n.s.
Ergosterol (μg ergosterol g^{-1})	65.6	61.6	79.1	0.637	n.s.
N-mineralisation (μg N g^{-1} 7 d^{-1})	134.1	127.5	155.8	1.132	n.s.
Protease (μg tyrosine g^{-1} 2 h^{-1})	2974 a	4803 b	2844 a	11.179	**
Arginine deaminase (μg N g^{-1} 2 h^{-1})	29.6	53.2	32.1	2.895	n.s.
Urease (μg N g^{-1} 2 h^{-1})	289.5	332.3	333.2	1.107	n.s.

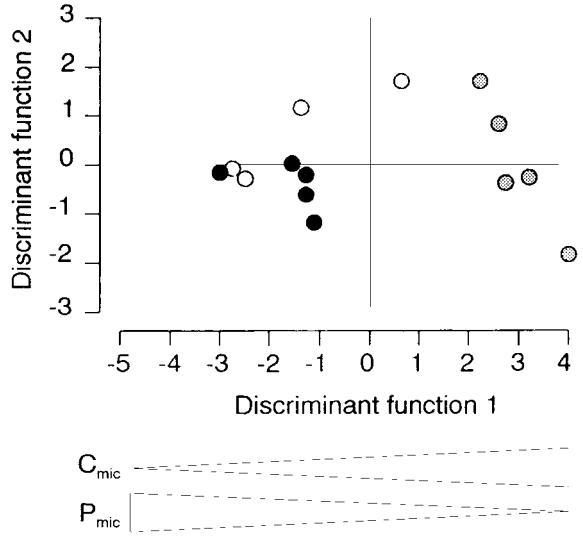

Fig. 1. Discriminant analysis based on the biomass variables (SIR, C_{mic}, N_{mic} and P_{mic} and ergosterol content) from the L/F layer of mesocosms and undisturbed soil. Axis 1 is labelled by the most important parameters (C_{mic} and P_{mic}). Open circles: treatment 1 (mesocosms with microbiota), closed circles: treatment 2 (mesocosms with microbiota and fauna), grey circles: control (undisturbed soil). Treatment 1: $n = 4$, treatment 2 and control: $n = 5$.

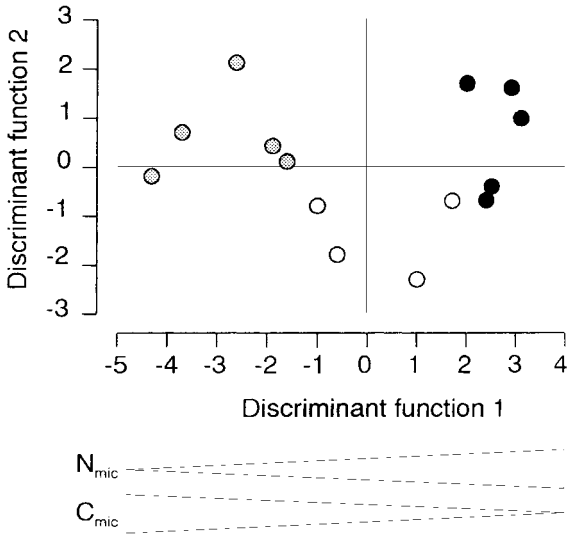

Fig. 2. Discriminant analysis based on the biomass variables (SIR, C_{mic}, N_{mic} and P_{mic} and ergosterol content) from the H layer of mesocosms and undisturbed soils. Axis 1 is labelled by the most important parameters. Open circles: treatment 1 (mesocosms with microbiota), closed circles: treatment 2 (mesocosms with microbiota and fauna), grey circles: control (undisturbed soil). Treatment 1: $n = 4$, treatment 2 and control: $n = 5$.

The re-immigration of mesofauna into the mesocosms had no effect on the C-to-N ratio and pH. The undisturbed soil had a significantly lower pH and lower ammonium contents than the two mesocosm treatments (Tables 2 and 3). Mesocosms with mesofauna contained a significantly higher amount of lactate-soluble P and showed a trend towards higher ammonium content in the H layer (Table 3).

3.3. Microbial biomass and community structure

In the L/F layer, univariate statistical analysis revealed that re-immigration of mesofauna into meso-

Table 4
Statistics of the discriminant analyses based on biomass and N-cycle variables in the L/F and H layer of mesocosms and undisturbed soil. Standardised discriminant coefficients for the biomass and N-cycle variables are only presented for significant discriminant functions ($^*p < 0.05$)

Layer	Variable complex	Discriminant function	% of variance	Wilks' L	χ^2	d.f.	p	Variables	Standardised discriminant coefficients
L/F	biomass	1	96.6	0.118	19.22	10	0.038*	SIR	−0.077
								C_{mic}	1.422
								N_{mic}	−0.381
								P_{mic}	−1.337
								ergosterol	0.808
		2	3.4	0.827	1.71	4	0.790		
	N-cycle	1	98.7	0.257	12.9	8	0.115		
		2	1.33	0.964	0.35	3	0.951		
H	biomass	1	87.3	0.067	24.34	10	0.007*	SIR	−0.482
								C_{mic}	−1.657
								N_{mic}	1.974
								P_{mic}	0.982
								ergosterol	−0.671
		2	12.7	0.510	6.06	4	0.195		
	N-cycle	1	98.2	0.063	26.26	8	0.001*	protease	1.139
								urease	0.489
								arginine deaminase	1.397
								N-mineralisation	−2.126
		2	1.9	0.816	1.93	3	0.587		

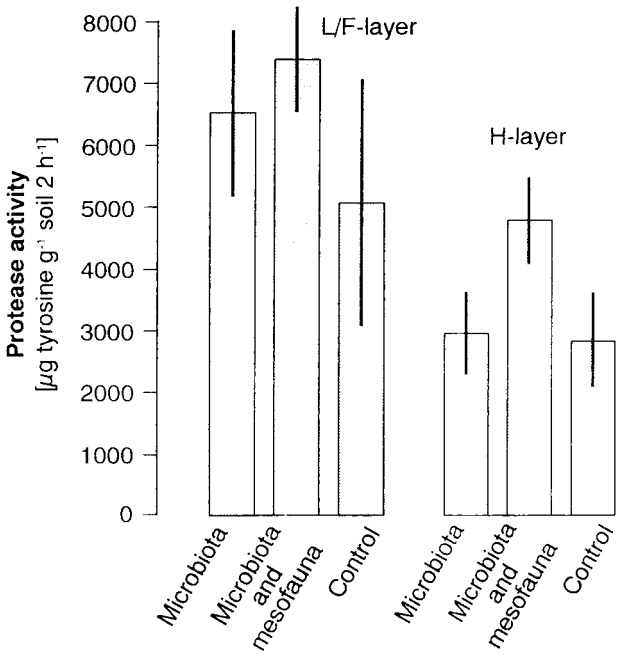

Fig. 3. Protease activity of the L/F and H layers in mesocosms and controls in a spruce forest after exposure for 6 months. Results are given as means of five replicates and standard deviation.

cosms did not significantly affect SIR, biomass C, biomass N, biomass P or ergosterol content (Table 2). The biomass C and ergosterol contents were greater in the control than in either mesocosm, whereas SIR, biomass N and biomass P did not differ between the three treatments (Table 2). The discriminant analysis of SIR, biomass C, biomass N, biomass P and ergosterol content clearly separated the control (undisturbed soil) and the mesocosms, irrespective of mesocosm type, along discriminant axis 1 (Fig. 1). The discrimination along axis 1 was due to an increase of biomass C and a decrease of biomass P. Discriminant function (DF) 1 explains more than 97% of the total variance of the data set (Table 4).

In the H horizon, SIR, biomass C, biomass N, biomass P and ergosterol content showed a trend towards higher values under the influence of the mesofauna, but the univariate statistical analysis failed to detect significant differences between the two treatments (Table 3). Including the data set in a discriminant function, statistical analysis revealed that DF 1 explained 87% of the total variance of the data set and was dominated by biomass N and biomass C, which had the highest standardised coefficients in the linear combination (Fig. 2, Table 4). The discriminant functions were very powerful in identifying the different treatments in the H horizon, except in one mesocosm with microbiota only.

The content of ester-linked phospholipid fatty acids (EL-PLFAs) in the L/F layer was in the range of 1108

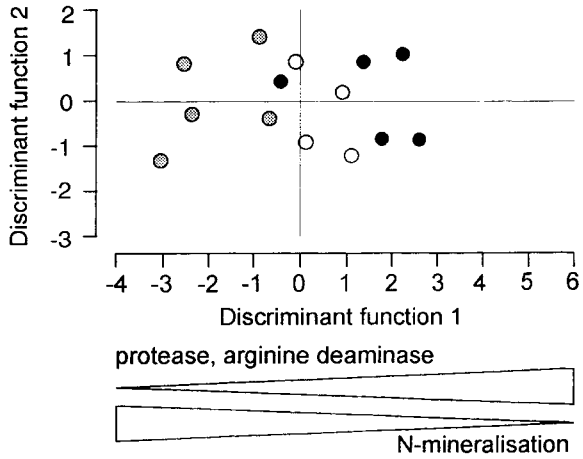

Fig. 4. Discriminant analysis based on the N-cycle variables (N-mineralisation, protease, arginine deaminase and urease) from the L/F layer of mesocosms and undisturbed soils. Axis 1 is labelled by the most important parameters. Open circles: treatment 1 (mesocosms with microbiota), closed circles: treatment 2 (mesocosms with microbiota and fauna), grey circles: control (undisturbed soil). Treatment 1: $n = 4$, treatment 2 and control: $n = 5$.

to 1500 nmol g^{-1} (Table 1). These amounts corresponded well with amounts in the OF horizon of a podsolic soil under *Picea abies* (Zelles et al., 1995). Satured fatty acids (SATFA) were the dominant fraction in the L/F layer, yielding about 50% of the total content of EL-PLFAs, followed by the mono-unsaturated fatty acids (MUFAS, 27%), hydroxy-substituted fatty acids (PLOHS, 12%) and the polyunsaturated fatty acids (PUFAS 8%). The total content of EL-PLFAs of the L/F layer as well as the LF specific for fungi (PLFA 18:2ω6) and bacteria (sum of 12 different

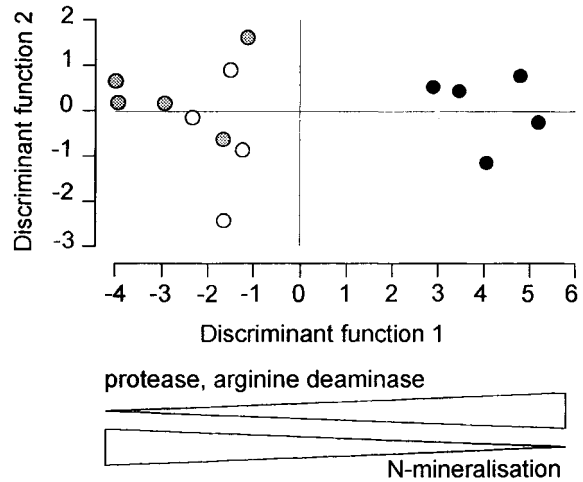

Fig. 5. Discriminant analysis based on the N-cycle variables (N-mineralisation, protease, arginine deaminase and urease) from the L/F layer of mesocosms and undisturbed soils. Axis 1 is labelled by the most important parameters. Open circles: treatment 1 (mesocosms with microbiota), closed circles: treatment 2 (mesocosms with microbiota and fauna), grey circles: control (undisturbed soil). Treatment 1: $n = 4$, treatment 2 and control: $n = 5$.

fatty acids) were not affected by the re-immigration of mesofauna into the mesocosms (Table 2). Hierarchical cluster analysis including all PLFA's revealed that it is not possible to distinguish the three treatments on the basis of their phospholipid fatty acid profiles in the L/F horizon (data not shown).

3.4. Decomposition of organic nitrogen

Rates of N-mineralisation and enzymes involved in nitrogen cycling (protease, urease and arginine deaminase) were not affected by mesofauna in the L/F horizon (Table 2). In addition, these microbiological properties did not differ between mesocosms and control. In contrast, the activities of protease, urease and arginine deaminase in the H layer showed a trend towards higher activity in mesocosms with higher faunal complexity. This, however, was only significant for protease activity (Fig. 3, Table 3).

The discriminant analysis including N-mineralisation and enzymes involved in N cycling in the L/F layer did not yield any significant discriminant function (Fig. 4). In contrast, there was a significant discrimination between mesocosms with microbiota only and mesocosms with microbiota and mesofauna along discriminant axis 1 in the H layer (Fig. 5). Discriminant function (DF) 1 explained 98.2% of the total variance of the data set and was dominated by N-mineralisation, protease and arginine deaminase, which had the highest standard coefficients in the linear combination (Table 4). Because of the high eigenvalue of DF 1, these variables were the most important in relation to all other discriminant functions. DF 2 explained only 1.9% of the total variance and did not significantly contribute to the discrimination of the data. Classification according to discriminant functions revealed that 3 of 14 objects were misclassified. It was not possible to separate mesocosms including only microbiota from controls (Fig. 5).

4. Discussion

4.1. Chemical properties

The mesocosm approach provides the opportunity to investigate microbial–faunal interactions that reflect those in the natural ecosystem (Kampichler et al., 1999). Our results clearly showed that mesocosms and control (undisturbed soil) differed with respect to water content and pH. The enclosing nets used to control the re-immigration of certain body size classes of animals into the mesocosms also prevented root growth into the mesocosms. Therefore, the lack of water uptake by roots might have caused the lower water content in the mesocosms in comparison to the control. Similar results were obtained in an earlier mesocosm experiment (Kandeler et al., 1994). The different pH values in mesocosms and the control may also have been due to the prevention of root growth into the mesocosms, since nutrient uptake by plants is accompanied by a release of protons (Marschner, 1995). Since soil moisture, structural properties and pH affect the distribution and feeding activities of invertebrates, it may be impossible to simulate natural ecosystem conditions using mesocosms which lack plants. Mesofauna had a slight positive influence on the release of phosphate and ammonium in the H horizon. This finding is consistent with other studies that have reported accelerated P-mineralisation in microcosms and mesocosms (Anderson and Ineson, 1984; Zechmeister-Boltenstern et al., 1998). The enhanced P-mineralisation was found to be accompanied by a reduced microbial community and a subsequent reduction in microbial immobilisation (Setälä et al., 1990). Since microbial biomass in our experiment did not change significantly due to re-immigration of the mesofauna in the mesocosm, it is unlikely that the P release was caused by faunal grazing. An alternative explanation might be the cycling outside the microbial pathway, as reported by Setälä et al. (1990).

4.2. Microbial biomass and community structure

In aboveground systems grazing by herbivores increases plant diversity by maintaining microsites and reducing dominant competitors (McLean et al., 1996). In contrast, grazing on fungi by mites and collembolans did not affect the fungal community structure of pine needle litter in a microcosm experiment (McLean et al., 1996). In our experiment, univariate statistical analyses could not detect any significant influence of mesofauna on biomass C, N and P in L/F and H layers. However, the discriminant analysis with biomass data from the H layer indicated different contents of microbial biomass C and N in the two mesocosm treatments (Fig. 3). Therefore, the mesofauna induced an alteration of the C-to-N content of microbial populations, as described by Verhoef and Brussaard (1990). Insight into the soil microbial community was obtained by fungal biomass estimates based on the amount of the PLFA 18:2ω6 and the ergosterol content. Both estimates revealed that re-immigration of mesofauna into mesocosms did not change the fungal biomass in the litter layer. According to Federle (1986), who examined the content of 18:2ω6 in 47 species of soil fungi, this PLFA constituted about 43% of total PLFAs, but linoleic acid is also known to occur as a fraction of all fatty acids in *Pinus sylvestris* and *Picea. abies* (Saranpää and Nyberg, 1987; Wellburn et al., 1994; Tillman-Sutela et al., 1996). Until now it has not been well documented whether

PLFA 18:2ω6 as a component of the litter is rapidly decomposed in forest soils (Fritze and Bååth, 1993). Therefore, the effect of soil animals on this PLFA might be masked by an unknown amount of PLFA 18:2ω6 derived from plant material. Beyond this methodological aspect, the view that mesofauna did not shift the soil microbial community is supported by the cluster analysis including the single fatty acids which did not detect any differences due to mesofauna. Also, the effect of mesofauna may depend on the densities of soil fauna. The mesocosm technique used field densities of mesofauna. Collembolans at field density were not found to influence the biomass of the saprotrophic fungus *Phoma exigua* in microcosms containing litter from tussock grassland (Bardgett et al., 1993).

4.3. Decomposition of organic nitrogen compounds

Enzyme activities of the mesocosms were in the range previously reported for soils of spruce forests (Hamm and Feger, 1996; Vedder et al., 1996). As for chemical properties and soil microbial biomass, mesofauna changed the decomposition of organic nitrogen compounds in the H layer only. The enhanced production of protease appears to be a physiological response to faunal grazing. This hypothesis is supported by a microcosm experiment of Hedlund et al. (1991) which showed higher extracellular protease production of *Mortierella isabellina* by the grazing of *Onychiurus armatus* (Collembola) and by an earlier mesocosm experiment in a spruce forest (Kandeler et al., 1994). No effect of microarthropods on protease activity was found by Schlatte et al. (1998) in a microcosm experiment using comparable substrates, but lasting for a shorter period. Therefore, the duration of the experiment may strongly influence the effects of mesofaunal grazing activities on microbial biomass as well as on soil enzyme activities (Faber and Verhoef, 1991; Leonard and Anderson, 1991).

Discriminant analysis showed that mesofauna affected N-mobilisation in the H layer. The discrimination was due to an inverse trend of protease and arginine activities and of N-mineralisation. Mesofauna and macrofauna can modify nitrogen turnover of soils by direct and indirect mechanisms (Anderson et al., 1983, 1985; Abrahamsen, 1990; Setälä et al., 1990; Faber and Verhoef, 1991; Vedder et al., 1996). The trend towards higher ammonium contents at a constant N-mineralisation (measured by the incubation of soils under water-logged conditions) may have been caused by direct effects due to mineral nutrients in urine and faeces as suggested by Lussenhop (1992). In contrast, higher protease and arginine deaminase activities in mesocosms with microbiota and mesofauna presumably resulted from a physiological response of soil microorganisms to grazing by mesofauna.

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References

Abrahamsen, G., 1990. Influence of *Cognetia shagnetorum* (Oligochaeta: Enchytraeidae) on nitrogen mineralization in homogenized mor humus. Biology and Fertility of Soils 9, 159–162.

Alef, K., Kleiner, D., 1986. Arginine ammonification, a simple method to estimate microbial activity potentials in soils. Soil Biology & Biochemistry 18, 233–235.

Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10, 215–221.

Anderson, J.M., Ineson, P., 1984. Interaction between microorganisms and soil invertebrates in nutrient flux pathways of forest ecosystems. In: Anderson, J.M., Rayner, A.D.M., Walton, D.W.H. (Eds.), Invertebrate–Microbial Interactions. Cambridge University Press, Cambridge, pp. 59–88.

Amato, M., Ladd, J.N., 1988. Assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soils. Soil Biology & Biochemistry 20, 107–114.

Anderson, J.M., Huish, S.A., Ineson, P., Leonard, M.A., Splatt, P.R., 1985. Interactions of invertebrates, microorganisms and tree roots in nitrogen and mineral element fluxes in deciduous woodland soils. In: Fitter, A.H., Atkinson, D., Read, D.J., Usher, M.B. (Eds.), Ecological Interactions in Soil: Plants, Microbes and Animals. Blackwell Scientific Publications, Palo Alto, pp. 377–392.

Anderson, J.M., Ineson, P., Huish, S.A., 1983. Nitrogen and cation mobilization by soil fauna feeding on leaf litter and soil organic matter from deciduous woodland. Soil Biology & Biochemistry 15, 463–467.

Bardgett, R.D., Whittaker, J.B., Frakland, J.C., 1993. The effect of collembolan grazing on fungal activity in differently managed upland pastures: a microcosm study. Biology and Fertility of Soils 16, 255–262.

Bengtsson, G., Hedlund, K., Rundgren, S., 1993. Patchiness and compensatory growth in a fungus–Collembola system. Oecologia 93, 296–302.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biology & Biochemistry 14, 319–329.

Bruckner, A., Kampichler, C., Wright, J., Bauer, R., Kandeler, E., 1995. A method to prepare mesocosms to investigate mesofaunal–microbial interactions in soil. Biology and Fertility of Soils 19, 257–262.

Coleman, D.C., 1986. The role of microfloral and faunal interactions in affecting soil processes. In: Mitchell, M.J., Nakas, J.P. (Eds.), Microfloral and Faunal Interactions in Natural and Agro-ecosystems. Martinus Nijhoff/Dr. W. Junk, Dordrecht, Netherlands, pp. 317–348.

Djajakirana, G., Joergensen, R.G., Meyer, B., 1996. Ergosterol and microbial biomass relationships in soil. Biology and Fertility of Soils 22, 299–304.

Faber, J.H., Verhoef, H.A., 1991. Functional differences between closely related soil arthropods with respect to decomposition processes in the presence or absence of pine tree roots. Soil Biology & Biochemistry 23, 15–23.

Federle, T.W., 1986. Microbial distribution in soil — new techniques. In: Megusar, F., Gantar, M. (Eds.), Perspectives in Microbial Ecology. Slovene Society for Microbiology, Ljubljana, pp. 493–498.

Förster, B., Römbke, J., Knacker, T., Morgan, E., 1995. Microcosm study of the interaction between microorganisms and enchytraeid worms in grassland soil and litter. European Journal of Soil Biology 31, 21–27.

Fritze, H., Bååth, E., 1993. Microfungal species composition and fungal biomass in a coniferous forest soil polluted by alkaline deposition. Microbial Ecology 25, 83–92.

Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59–65.

Hamm, D., Feger, K.H., 1996. Eine optimierte Methode zur Bestimmung der Proteaseaktivität in sauren Waldböden. Zeitschrift fuer Pflanzenernaehrung und Bodenkunde 159, 37–39.

Hedlund, K., Boddy, L., Preston, C.M., 1991. Mycelial responses of the soil fungus, *Mortierella isabellina*, to grazing by *Onychiurus armatus* (Collembola). Soil Biology & Biochemistry 23, 361–366.

Hoffmann, G., Ohnesorge, S., 1966. Bestimmung der Phosphorsäure in Bodenextrakten mit Ascorbinsäure Zinnchlorid als Reduktionsmittel. Landwirtschaftliche Forschung 19, 94–107.

Jäggi, W., 1976. Die Bestimmung der CO_2-Bildung als Mass der bodenbiologischen Aktivität. Schweizer Landwirtschaftliche Forschung 15, 371–380.

Joergensen, R.G., 1995a. The fumigation extraction method to estimate soil microbial biomass: extraction with 0.01 M $CaCl_2$. Agribiological Research 48, 319–324.

Joergensen, R.G., 1995b. Fumigation extraction method for microbial biomass phosphourus. In: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, pp. 389–391.

Joergensen, R.G., Brookes, P.C., 1990. Ninhydrin-reactive nitrogen measurements of microbial biomass in 0.5 M K_2SO_4 soil extracts. Soil Biology & Biochemistry 22, 1023–1027.

Jongman, R.H., ter Braak, C.J.F., van Tongeren, O.F.R., 1996. Data Analysis In Community and Landscape Ecology. Cambridge University Press, Cambridge.

Kamipchler, C., Kandeler, E., 1996. Microbial–faunal interactions in soil. In: Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R. (Eds.), Methods in Soil Biology. Springer-Verlag, Berlin, pp. 364–382.

Kampichler, C., Bruckner, A., Baumgarten, A., Bertold, A., Zechmeister-Boltenstern, S., 1999. Field mesocosms for assessing biotic processes in soils: how to avoid side effects. Science of Soils (submitted for publication).

Kandeler, E., Gerber, H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biology and Fertility of Soils 6, 68–72.

Kandeler, E., Winter, B., Kampichler, C., Bruckner, A., 1994. Effects of mesofaunal exclusion on microbial biomass and enzymatic activities in field mesocosms. In: Ritz, K., Dighton, J., Giller, K. (Eds.), Beyond the Biomass. Wiley and Sons, Chichester, pp. 181–189.

Klironomos, J.N., Widden, P., Deslandes, I., 1992. Feeding preferences of the collembolan *Folsomia candida* in relation to microfungal successions in decaying litter. Soil Biology & Biochemstry 24, 685–692.

Ladd, J.N., Butler, J.H.A., 1972. Short-term assay of soil protolytic enzyme activities using proteins and dipeptide derivates as substrates. Soil Biology & Biochemistry 4, 19–30.

Leonard, M.A., Anderson, J.M., 1991. Growth dynamics of collembola (*Folsomia candida*) and a fungus (*Mucor plumbeus*) in relation to nitrogen availability in spatially simple and complex laboratory systems. Pedobiologia 35, 163–173.

Lussenhop, J., 1992. Mechanisms of microarthropod–microbial interactions in soil. Advances in Ecological Research 23, 1–33.

Lussenhop, J., 1996. Collembola as mediators of microbial symbiont effects upon soybean. Soil Biology & Biochemstry 28, 363–369.

Marschner, H., 1995. Mineral Nutrition of Higher Plants, 2nd ed. Academic Press, London.

McLean, M.A., Kaneko, N., Parkinson, D., 1996. Does selective grazing by mites and collembola affect litter fungal community structure? Pedobiologia 40, 97–105.

Morris, A.W., Riley, J.P., 1963. The determination of nitrate in sea water. Analytica Chimica Acta 29, 272–279.

Newell, K., 1984. Interaction between two decomposer basidiomycetes and a collembolan under Sitka spruce: grazing and its potential effects on fungal distribution and litter decomposition. Soil Biology & Biochemistry 16, 235–239.

Saranpää, P., Nyberg, H., 1987. Lipids and sterols of *Pinus sylvestris* L. sapwood and heartwood. Trees 1, 82–87.

Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R., 1996. Methods in Soil Biology. Springer-Verlag, Berlin.

Schlatte, G., Kampichler, C., Kandeler, E., 1998. Do soil microarthropods influence microbial biomass and activity in spruce forest litter? Pedobiologia 42, 205–214.

Setälä, H., Martikainen, E., Tyynismaa, M., Huta, V., 1990. Effects of soil fauna on leaching of nitrogen and phosphorus from experimental systems simulating coniferous forest floor. Biology and Fertility of Soils 10, 170–177.

Tillman-Sutela, E., Johansson, A., Kauppi, A., Laakso, P., Kallio, H., 1996. Lipids and their seasonal variation in the nucellar layers of Scot pine (*Pinus sylvestris*) seeds. Canadian Journal of Botany 74, 1416–1424.

Tunlid, A., White, D.C., 1992. Biochemical analysis of biomass, community structure, nutritional status and metabolic activity of microbial communities in soil. In: Stotzky, G., Bollag, J.M. (Eds.), Soil Biochemistry, Vol. 7. Marcel Dekker, New York, pp. 229–262.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19, 703–707.

Vedder, B., Kampichler, C., Bachmann, G., Bruckner, A., Kandeler, E., 1996. Impact of faunal complexity on microbial biomass and N turnover in field mesocosms from a spruce forest soil. Biology and Fertility of Soils 22, 22–30.

Verhoef, H.A., Brussaard, L., 1990. Decomposition and nitrogen mineralization in natural and agroecosystems: the contribution of soil animals. Biogeochemistry 11, 175–211.

Verhoef, H.A., de Goede, R.G.M., 1985. Effects of collembolan grazing on nitrogen dynamics in a coniferous forest. In: Fitter, A.H., Atkinson, D., Read, D.J., Usher, M.B. (Eds.), Ecological Interactions in Soil: Microbes and Animals. Blackwell, Palo Alto, pp. 297–317.

Wellburn, A.R., Robinson, D.C., Thomson, A., Leith, I.D., 1994. Influence of episodes of summer O_3 on $\Delta 5$ and $\Delta 9$ fatty acids in automnal lipids of Norway spruce (*Picea abies*). New Phytologist 127, 355–361.

Zechmeister-Boltenstern, S., Baumgarten, A., Bruckner, A., Kampichler, C., Kandeler, E., 1998. Impact of faunal complexity on nutrient supply in field mesocosms from a spruce forest soil. Plant and Soil 198, 45–52.

Zelles, L., Bay, Q.Y., 1993. Tractionation of fatty acids derived from soil lipids by solid phase extraction and their quantitative analysis by GC-MS. Soil Biology & Biochemistry 25, 459–507.

Zelles, L., Bai, Q.Y., Rackwitz, R., Chadwick, D., Beese, F., 1995. Determination of phospholipid- and lipopolysyccharide-derived fatty acids as an estimate of microbial biomass and community structures in soils. Biology and Fertility of Soils 19, 115–123.