Working Report 2007-08

Dissolved Nitrogen Transformations and Microbial Community Structure in the Organic Layer of Forest Soils in Olkiluoto in 2006

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ABSTRACT

Carbon (C) and nitrogen (N) cycles in the ecosystem are strongly coupled. Biomass, structure and activity of the bacterial and fungal community are the key factors influencing C and N cycles. Changes in the function of soil microbial community can be a signal of plant responses to environmental changes. Dissolved N compounds, microbial biomass, microbial activity, fungal community structure and functional diversity of microbial communities were measured in September 2006 from five monitoring plots on Olkiluoto to assess information about soil microbial community structure and activity. High within and between variation in the studied plots were detected. However, in this study the values and their variation in the level of N mineralisation, dissolved N compounds, fungal biomass and microbial community structure in the studied plots were within a normal range in comparison with other published data of similar forest types in Finland.

Keywords: Community level physiological profile, Dissolved nitrogen, Dissolved organic carbon, Ergosterol, Fungal community structure, Microbial biomass, Mineralisation

LIUKOISET TYPPIYHDISTEET, MINERALISAATIO JA MIKROBIYHTEISÖN RAKENNE METSÄMAAN ORGAANISESSA KERROKSESSA OLKILUODOSSA 2006

TIIVISTELMÄ

Hiilen (C) ja typen (N) kierrot ekosysteemissä ovat tiiviissä yhteydessä toisiinsa. Bakteeri- ja sieniyhteisön biomassa, rakenne ja aktiivisuus ovat keskeisessä asemassa C ja N kierrossa. Muutokset maaperän mikrobiyhteisön toiminnassa voivat heijastaa kasviyhteisön vastetta ympäristömuutoksiin. Liukoiset N-yhdisteet, mikrobibiomassa, mikrobiaktiivisuus, sieniyhteisön rakenne ja mikrobiyhteisön toiminnallinen diversiteetti mitattiin syyskuussa 2006 viideltä seurantakoealalta Olkiluodossa maaperän mikrobiyhteisön rakenteen ja toiminnan arvioimiseksi. Havaintoalojen sisäinen ja niiden välinen vaihtelu oli runsasta. Kuitenkin havaitut arvot ja niiden vaihtelu typen mineralisaatiossa, liukoisissa typpiyhdisteissä, sienibiomassassa ja mikrobiyhteisön rakenteessa vastasi muualta Suomesta saman tyyppisistä metsistä tehtyjä havaintoja.

Avainsanat: ergosterol, liukoinen typpi, liukoinen orgaaninen hiili, mikrobibiomassa, mineralisaatio, sieniyhteisön rakenne, yhteisötason fysiologinen profiili

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DEFINITIONS

AWCD	Average well colour development
CLPP	Community level physiological profile
DGGE	Denaturing gradient gel electrophoresis
ectomycorrhiza	Mycorrhizal type in which the fungal mycelia extend inward, between root cortical cells, to form a network (Hartig net) and outward into the surrounding soil. Fungi produce this kind of mycorrhiza with the main tree species in Finland.
ergosterol	Major sterol of the plasma membranes of fungi.
eukaryotic	Organism having a unit membrane-bound nucleus and usually other organelles.
extramatrical	Hyphal strands that extend beyond the root mantle into the soil mycelium matrix (synonym: external mycelia).
heterotroph	Organism capable of deriving carbon for growth and cell synthesis from organic compounds.
incubation	Sample is kept at constant temperature for a specific period of time.
ITS fragments	DNA fragment from internal transcribed spacer region.
ITS region	The internal transcribed spacer (ITS) region contains two variable non-coding regions that are nested within the ribosomal DNA repeat between the highly conserved small subunit, 5.8S, and large subunit ribosomal DNA genes.
mycelium	Mass of hyphae that form the vegetative body of many fungal organisms.
mycorrhizosphere	Zone of soil immediately adjacent to mycorrhizal fungal mycelia.
prokaryotic	Organism lacking a unit membrane-bound nucleus and other organelles, usually having its DNA in a single circular molecule.
rhizosphere	Zone of soil immediately adjacent to plants roots in which the kind, numbers, or activities of microorganisms differ from those in the bulk soil.

saponification	The hydrolysis of an ester under basic conditions to form an alcohol and the salt of the acid.
saprophytic	Nonparasitic nutritional mechanism by which an organism obtains its nutrients exclusively from the degradation of nonliving organic material.
sequencing	Determine the primary structure of an unbranched biopolymer. DNA sequencing in the process of determining the nucleotide order of a given DNA fragment.
sporocarp	A specialised type of fruiting body, the primary function of which is the production and release of spores. In fungi, the large (visible) sporocarps occurring above the ground are generally called mushrooms, while those occurring below the ground are usually called truffles.

1 INTRODUCTION

Carbon (C) and nitrogen (N) cycles in ecosystems are strongly coupled. The decomposition of organic matter and assimilation of carbon in photosynthesis are the main processes in the biological carbon cycle, whereas mineralisation, nitrification and denitrification are the main processes in the soil nitrogen cycle. The biomass, structure and activity of the bacterial and fungal community are the key factors influencing C and N cycles.

Nitrogen can be present in various forms: dinitrogen gas (N_2), organic nitrogen, ammonium (NH_4^+) and nitrate (NO_3^-). Microbial processes transform N from one form to another. Mineralisation refers to the conversion of an element from an organic form to an inorganic state as a result of microbial decomposition (Sylvia et al. 1999). Thus, nitrogen mineralisation is the production of inorganic N from organic forms. It can be divided into ammonification and nitrification. Ammonification means the biological transformation of organic nitrogen to ammonium, and nitrification the microbial production of nitrate by autotrophic or heterotrophic processes. Autotrophic nitrification is the biological oxidation of ammonium to nitrite and nitrate. In heterotrophic nitrification, micro-organisms oxidise either ammonium or organic nitrogen to nitrite or nitrate. Denitrification is an opposite process to nitrification, and produces the gases molecular nitrogen (N_2) or nitrogen oxides (NO_x).

The free-living microbial community in soil is composed of bacteria, fungi and algae populations. The role of the soil microbial community and its processes as modifiers of ecosystem or plant responses to environmental changes is reviewed by Rilling et al. (2002). The response of plants to environmental changes can be partly due to changes in the functioning of the soil microbial community. The activity and population diversity of the microbial community are affected by environmental factors such as soil temperature, aeration and the ground water level. Changes in the functioning or diversity of the microbial community may have direct effects on nutrient cycling, including gas emissions, in forest ecosystems.

Microbial populations are responsible for the decomposition of organic material in the soil. Fungi are eukaryotic organisms that form the major part of the microbial biomass in boreal forests. In the soil, there are both saprophytic and mycorrhizal fungal mycelia. Fungi play an important role in nutrient cycling and decomposition processes in boreal forest ecosystems especially, where the low soil pH decreases bacterial activity. In boreal and temperate forests, trees live in symbiosis predominantly with ectomycorrhizal (ECM) fungi. The amount of extramatrical mycorrhizal mycelium (EMM) in the soil varies considerably and depends on the fungus species. The EMM is in close physical contact with the soil environment, and is thus subjected to chemical or physical changes in the soil. Furthermore, turnover of the mycelia is rapid, and thus changes in EMM may be a sensitive indicator of the ECM response to environmental disturbances (Erland & Taylor 2002).

Bacteria are single-celled, prokaryotic microbes which inhabit soils throughout the world in large numbers (Sylvia et al. 1999). They are much smaller than the eukaryotic microbes and fungi, and have a much simpler cellular structure. Most soil bacteria are heterotrophs,

and they can grow in the presence or absence of oxygen. Bacteria colonize specific soil environments like particles, root surfaces, and the rhizosphere and mycorrhizosphere in great numbers.

According to Merilä (2002), the quality of the organic matter changes and mineral N availability decreases during forest succession along the Bothnian land-uplift coast. However, the microbial biomass and respiration are relatively stable among the successional forest sites, despite the clear differences in the structure of the microbial communities. Aikio et al. (2000) found an increase in basal respiration and microbial biomass along the primary succession gradient of a dry heath forest on Hailuoto Island. The soil microbial activity and biomass along the land-uplift coast appears to depend on the type and chemical composition of the soil arising from the sea, and on the developing plant community structure.

Olkiluoto Island, which is located on the postglacial land up-lift coast of the Gulf of Bothnia, has been selected as the final disposal site for spent nuclear fuel. An underground rock characterisation facility, "ONKALO", is currently being constructed on the island. Due to these ongoing anthropogenic activities, increasing attention has been paid to environmental monitoring in the area. In addition to the monitoring, the Finnish regulations give guidance to assess the safety of the disposal of spent nuclear fuel for a time period of at least several thousand years (STUK 2001). The initial phase of the work is to describe the current biosphere on Olkiluoto Island. The microbial community plays an important role in the element cycles of the biosphere, especially the C and N cycles, and may also in the geosphere-biosphere interface zone. Thus our results may give additional information about the processes involved in nutrient cycling in soil. Geochemistry, biomass and microbial diversity of shallow subsurface groundwater from 4.0 m down to 24.5 m at Olkiluoto have been characterised by Pedersen (2006).

In this case study we have quantified the biomass, activity, and structural and functional diversity of the microbial community in five monitoring plots on Olkiluoto Island. We have also assessed the within- and between-plot variation in order to evaluate the rate of microbial processes, as well as the structure of the fungal and bacterial community.

2 MATERIAL AND METHODS

2.1 Study area

Five plots were selected for the case study: stand throughfall monitoring plots (MRK) 1, 6 and 8, and forest intensive monitoring plots (FIP) 4 and 10 (Fig. 1). Element cycles are being continuously monitored on these plots. The plots also represent the predominant forest types on Olkiluoto Island (Rautio et al. 2004, Saramäki & Korhonen 2005). The general characteristics of the plots are presented in Table 1.

2.2 Sampling and soil properties

Samples of the organic layer were collected on 13 and 14 September 2006. Two sampling points per plot were located in undisturbed areas in the vicinity of each plot (Fig. 1), and represented typical conditions in the monitored forest stands. Ten organic layer sub-samples were taken within a 3 m radius at each sampling point with an auger (d = 10 cm) and combined to give one bulked sample. The thickness of the organic layer in each sub-sample was measured, and living plant material and underlying mineral soil were separated from the organic layer sample. If the thickness of the organic layer was over 10 cm (in 11 of the sub-samples), the sub-sample was taken only down to a depth of 10 cm. On plots MRK1 and FIP10 the thickness of the organic layer used in the calculations was 80 mm and 81 mm, respectively. On the other plots the organic layer thickness in all the sub-samples was less than 60 mm. The samples were taken to the laboratory and kept cool (4 ºC) until mixed and weighed.

To determine soil properties the moisture content (determined by drying overnight at 105 ºC), organic matter content (OM; 485 ºC, 4h) and pH (in 0.01 M $CaCl_2$ or water, 1:2.5 v/v, standing overnight) were determined. The samples were oven-dried (+ 60 °C) and homogenized. The carbon and nitrogen concentrations of the humus samples were analysed on a CHN analyser (Leco CHN-2000) in the laboratory of the Parkono Research Unit. The element concentrations (P, K, S, Ca, Mg, B, Cu, Zn, Mn, Na, Fe, Al, Cd, Cr, Ni, Pb and Mo) were determined by wet digestion (HNO_3 / H_2O_2) and analysed by ICP-AES in the Central Laboratory of the Finnish Forest Research Institute. Wet digestion was carried out in a microwave oven. The results were expressed on a dry matter basis (determined by drying at +105 °C).

2.3 Dissolved nitrogen and carbon compounds

Extractable dissolved N and C were determined by extracting 15 g of fresh sample with 150 ml of 0.5 M K_2SO_4 (2 h shaking), and filtering the suspension first through filter paper and then through a 0.45 µm membrane filter (Williams et al. 1995). The extract was frozen (-20 °C) until analysis. Total dissolved N (TDN), NH_4^+-N and (NO_2^-+NO_3^-)-N in the extracts were determined by flow injection analysis (FIA Star 5020, Tecator). Dissolved organic nitrogen (DON) in the extracts was obtained by subtracting the NH_4^+-N

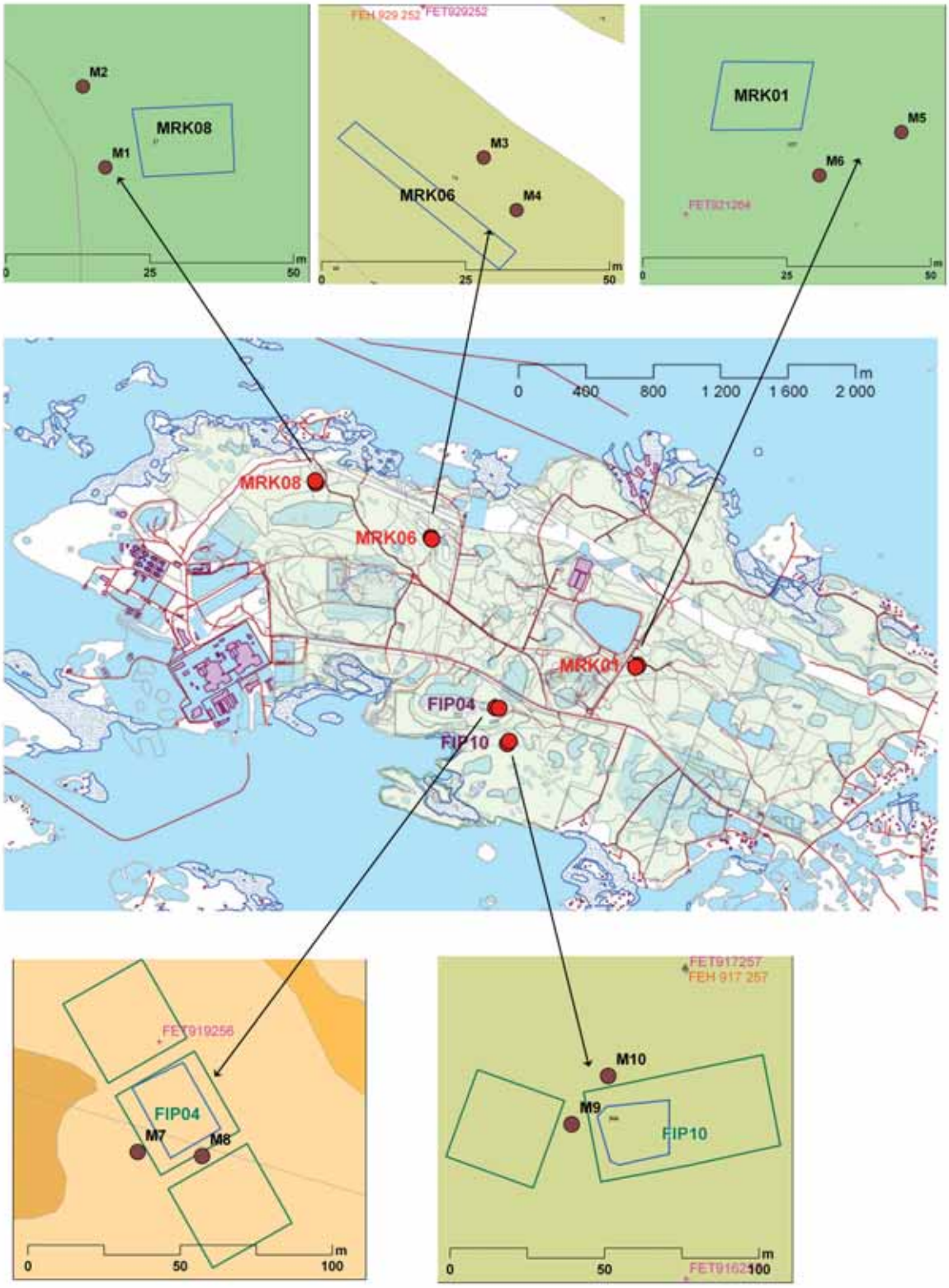

Figure 1. *Location of the study plots and sampling places at Olkiluoto.*

Table 1. *General characteristics of the plots. Forest site type and tree stand age according to Rautio et al. (2004). However the stand characteristics in Rautio et al. were determined on a larger compartment than the plots in this study.*

Plot	Sample number used in Appendices	Forest site type[1)]	Dominant tree species	Mean age, yrs (in 2003)
MRK8	1, 2	OMT	Norway spruce	80
MRK6	3, 4	MT	Norway spruce	35
MRK1	5, 6	MT	Scots pine	22
FIP4	7, 8	OMT	Scots pine	39
FIP10	9, 10	OMT	Norway spruce	91

[1)] MT, *Vaccinium myrtillus* type: fresh mineral soil site
OMT, *Oxalis acetosella- Vaccinium myrtillus* type: grove-like mineral soil site

and (NO_2^-+NO_3^-)-N concentrations from TDN. Dissolved organic carbon (DOC) in the extract was determined on a TOC analyser (Schimadzu 5000).

2.4 Mineralisation

Net N transformation was measured by excluding the N uptake of roots in the laboratory. Two replicate soil samples per plot were inserted in plastic bottles, closed at the top with loose lids. One of the replicate samples was extracted immediately, and the other one was incubated for 31 days at 14 °C in the dark in the laboratory, after which it was extracted. The moisture content of the samples was checked weekly, and water was added if necessary. The samples were extracted and analysed in the same way as the samples for the determination of dissolved N. Net ammonification and nitrification were calculated by subtracting the initial NH_4^+-N and NO_3^--N concentrations from the final NH_4^+-N and NO_3^--N concentrations at the end of the incubation period. Net N mineralisation was calculated as the sum of net ammonification and net nitrification. Negative values for this difference are referred to as net immobilisation (Williams 1992).

2.5 Microbial biomass

Ergosterol is a sterol component of fungal membranes, and it is assumed to be more strongly associated with living cells (Nylund & Wallander 1992), and thus may be a good estimate of living fungal biomass (Wallander et al. 1997). Fungi are more abundant, on a mass basis, in soils than any other group of microbes (Sylvia et al. 1999).

The ergosterol concentrations in the organic layer were determined by a method modified from Nylund and Wallander (1992), consisting of ethanol extraction, saponification with KOH and further extraction in pentane, followed by HPLC with a UV-VIS detector

(Merck-Hitatchi) (wavelength 280 nm), LiCrospher 100 RP-18 column and methanol as eluent. Microbial biomass C (C_{mic}) and N (N_{mic}) were determined from fresh organic layer samples by fumigation-extraction (Brookes et al. 1985) using a recovery factor of 0.45 for microbial C (Sparling et al. 1990) and 0.54 for microbial N (Brookes et al. 1985).

2.6 Fungal community structure

The individual components of the microbial population can be studied by molecular methods. Utilization of specific primers, for example ITS1F-ITS2, after DNA extraction from soil enables fungal populations to be studied separately from bacteria. With these methods it is possible to detect changes even at the species level following changes in the environmental conditions.

DNA was extracted from 280–300 mg (fresh weight) organic layer samples using the procedures described by Vainio and Hantula (2000). Briefly, this involved the addition of 200 µl H_2O to 250–300 mg of organic sample in Bead Solution tubes (Ultra clean Soil DNA Isolation Kit, MoBio Laboratories Inc., Carlsbad, California, USA). The samples were then lysed for 3 x 20 s at a speed of 4 m s^{-1} in a Fast Prep bead beating system and by heating at 65 ˚C for 30 min. Otherwise, the DNA extraction procedure for all the samples followed the instructions of the manufacturer. Samples were further purified with phenol/chloroform/isoamyl alcohol (59:49:1, by vol.) and with chloroform/isoamyl alcohol (24:1, by vol.). Purification was continued by adding 0.6 volume of polyethylene glycol in 2.5 M NaCl, incubating on ice, centrifugation and washing with 70% ethanol, drying and resuspending in TE-buffer. 50 µl of the aqueous phase was then subjected to separate polyvinylpolypyrrolidone (PVPP, Sigma) column purification as described by Pennanen et al. (2004).

The fungal ITS region was PCR amplified using the primers ITS1-F (Gardes & Bruns, 1993) and ITS2 (White et al. 1990). Because the yield of PCR amplification was low after the first amplification, we reproduced the amplification. A community fingerprinting technique called denaturing gradient gel electrophoresis (DGGE) was applied after PCR-amplification. DGGE analysis and sequencing were carried out as described by Korkama et al. (2006). Shortly, the amplification products of ITS1 were run in DGGE at a vertical gradient of 18–58%. Seven bands from the gel were excised for further re-run to a maximum of four times. The samples from single bands were amplified with an ITS1F-ITS2 primer pair and purified with High Pure PCR Product Purification Kit (Roche, Mannheim, Germany). Direct sequencing of the purified PCR samples was performed with a CEQ 8000 DNA analysis system and Quick Start Kit (Beckman Coulter Inc., Fullerton, California, USA).

2.7 Community level physiological profile of the microbes

The community level physiological profile (CLPP) describes the functional diversity of the microbial community. This can be studied by measuring the carbon utilization patterns of the microbial population.

CLPP was studied by Biolog Ecoplates (Biolog Inc. Hayward; California, USA). Each of the 96 wells of an EcoPlate contained dried nutrient solutions, a single carbon (C) compound, and a redox-colorant (tetrazolium violet) (three replicate wells for each of the 31 C sources and control without C). The CLPPs of the microbial communities were determined by extracting 10 g of fresh organic layer with 100 ml of Ringer solution (Oxoid) for 10 min with shaking. The samples were centrifuged twice at 720 x g for 10 min periods and the supernatant was diluted 1:10, 1:100 and 1:1000 to obtain more detailed information about the dilution effect on the CLPP of the microbial community. 150 µl of sample was added to each of the 96 wells in the EcoPlates (one EcoPlate per dilution per plot). The plates were incubated at 20 °C and colour development was read first at 0 h, and then daily as the optical density (OD) at 590 nm with a Labsystems Multiskan RC reader. The plates were measured until their average well colour development (AWCD) was 0.6 absorbance units or had begun to decrease.

2.8 Data analysis

The results are presented as average and standard error (SE) of concentrations per organic matter (OM) and on an area basis per m^2 in the studied organic layer.

All the bands (n = 38) in the DGGE gels were identified by gel analysis software Gene Tools (Syngene), and all the sample lanes were screened for the presence (1) or absence (0) of the bands, yielding a binary matrix. The similarity index of the plots was calculated between two samples per plot and between plots of each tree species. The methods used were the Jaccard (C_j) and Sörensen (C_s) indices. To emphasize common ITS fragments we eliminated those ITS fragments with fewer than two observations (15 ITS-fragments). This matrix (23 ITS fragments) was analysed by a non-metric multidimensional scaling (MDS) method by PC-ORD in order to identify differences in fungal community between and within the plots. MDS reduces complex DGE patterns to a point in a two-dimensional space by connecting the consecutive points. Thus, the relative changes in the microbial community can be visualized.

The sequences were manually aligned and checked using the BioEdit Sequence Alignment Editor and compared with the sequences in GenBank using BLASTN software.

The data from EcoPlates were calculated using the average well colour development (AWCD) (Garland & Mills 1991). Raw OD data were corrected by blanking each response well against its own first reading (0 h). AWCD was calculated for each microplate as $AWCD = \Sigma(A_j - A_k) / 31$, where A_j is the raw average absorbance in three wells of each substrate and A_k is the average absorbance in the control wells. Transformed data were calculated by dividing the corrected raw OD data value for each well by the AWCD of the plate. Values of less than 1.0 indicated a colour response less than the AWCD, and values over 1.0 indicated colour response greater than the AWCD (Garland & Mills 1991). Fingerprinting of the CLPP from the correlation matrix by principal component analysis (PCA) was performed separately for each dilution (1:10. 1:100, 1:1000) at AWCD 0.6. PCA is an ordination method that generates new variables, called principal components, which explain the highest dispersion of the samples. Diversity parameters, evenness (E)

and the Shannon – Wiener (H) diversity index, were calculated to evaluate microbial community functional diversity. The Shannon – Wiener diversity index (H) indicates a predominance of carbon sources in CLPP analysis, and it is lower when only one or a few carbon compounds show stronger dominance in the sample as a C source utilized by microbes (Legendre & Legendre 1998). If all C sources are utilized with the same intensity, then the value of evenness (E) is one.

3 RESULTS

3.1 Chemical properties of the organic layer

The thickness of the organic layer varied from 3 cm in plot FIP4 to 9 cm in plot FIP10 (Table 2). Soil pH ($CaCl_2$) varied from 3.5 to 3.7 and pH (H_2O) from 4.0 to 4.2. The C/N ratio varied from 22.3 to 30.5. The iron (Fe) concentrations in the two samples from plot MRK6, and in one sample from plots MRK1 and FIP10, were very high compared to the values for the other plots (Table 2, Appendix 1). Manganese (Mn) concentrations were low in FIP10 and in one sample from plots MRK6 and MRK1, but high in FIP4 (Table 2, Appendix 1). The molybdenum (Mo) concentrations were under the limit of quantitation of the analytical equipment, except in one sample from plot FIP10 where the Mo concentration was 1.02 mg kg^{-1} OM.

3.2 Dissolved N and C compounds

The concentrations of extractable dissolved organic carbon (DOC) in the organic layer were higher in FIP4 than the other plots (Fig. 2). DOC varied from 0.8 to 1.6 mg g^{-1} OM, except in one sample from FIP4 where it was 3.3 mg g^{-1} OM (Appendix 2). On an area basis, DOC varied considerably between the plots (from 7.1 to 13.3 g m^{-2}) (Fig. 2): the smallest amount was 4.5 g m^{-2} in one sample from plot FIP4 and the largest 13.7 g m^{-2} in one sample from plot FIP10. The DOC concentration was higher under Scots pine than under Norway spruce (1.62 mg g^{-1} OM, 1.25 mg g^{-1} OM, respectively), whereas the opposite was true on an area basis (8.41 g m^{-2}, 9.78 g m^{-2}, respectively).

The concentrations of ammonium-N (NH_4^+-N) in the organic layer varied from 6 to 36 mg kg^{-1} OM, being the highest in MRK8 (Fig. 3). The NH_4-N concentration was less than 10 mg kg^{-1} OM in plots FIP 10 and MRK 1, whereas it was 62 mg kg^{-1} OM in one sample from plot MRK8 (Appendix 2). The proportion of dissolved inorganic N (NH_4^+-N) out of total dissolved N varied from 6% in plot FIP10 to 32% in plot MRK8. It was higher under spruce than under pine (15% and 11%, respectively). Nitrate (NO_3^-) concentrations were below the quantitation limit.

The concentrations of dissolved organic N (DON) were 100 mg kg^{-1} OM or less in MRK6, FIP10 and MRK1 (Fig. 4). In one sample from plot FIP4 the DON concentration was 212 mg kg^{-1} OM. On an area basis, the amount of DON varied from 0.5 to 0.9 g m^{-2} (Fig. 4). The concentration of DON was higher under pine than under spruce (119 mg kg^{-1}, 102 mg kg^{-1}, respectively), whereas the opposite was true on an area basis (0.64 g m^{-2}, 0.76 g m^{-2}, respectively).

3.3 Mineralisation

The concentrations of mineralised N after 31 days incubation varied from 9 mg to 124 mg kg^{-1} OM, which corresponded to 0.4 mg to 3.7 mg kg^{-1} OM per day (Fig. 5). There was high variation in net N mineralisation between the samples: the minimum was

Table 2. *Thickness of the organic layer, organic matter content (OM), pH, C/N ratio and total elemental concentrations in the organic layer of the five plots. Values are mean and SE in parentheses (n = 2).*

		MRK6	MRK8	FIP10	FIP4	MRK1
Thickness (cm)		4.1 (0.1)	5.0 (0.8)	9.0 (0.8)	3.0 (0.2)	8.8 (1.9)
OM %		81.6 (0.6)	81.6 (5.0)	85.5 (4.9)	78.0 (5.4)	70.9 (0.6)
pH	(H_2O)	4.0 (0.17)	4.2 (0.0)	3.9 (0.01)	4.0 (0.05)	4.1 (0.20)
pH	($CaCl_2$)	3.6 (0.19)	3.7 (0.03)	3.5 (0.02)	3.5 (0.09)	3.5 (0.23)
C to N ratio		22.3 (1.57)	28.9 (0.01)	23.8 (1.02)	30.5 (2.89)	26.5 (0.92)
Al	mg kg^{-1} OM	7798 (535)	3151 (1405)	6477 (2716)	2521 (399)	9931 (2478)
	kg ha^{-1}	363 (24.9)	147 (65.5)	302 (127)	117 (18.6)	463 (115)
B	mg kg^{-1} OM	5.2 (0.34)	9.8 (0.13)	6.9 (0.43)	7.3 (1.32)	5.7 (0.08)
	kg ha^{-1}	0.2 (0.02)	0.5 (0.01)	0.3 (0.02)	0.3 (0.06)	0.3 (0.00)
Ca	mg kg^{-1} OM	5082 (1202)	7320 (518)	5452 (1406)	5550 (910)	4766 (612)
	kg ha^{-1}	237 (56.0)	341 (24.1)	254 (66)	259 (42.4)	222 (28.5)
Cd	mg kg^{-1} OM	1.1 (0.17)	1.1 (0.13)	0.7 (0.12)	0.9 (0.12)	0.9 (0.19)
	kg ha^{-1}	0.05 (0.01)	0.05 (0.01)	0.03 (0.01)	0.04 (0.01)	0.04 (0.01)
Cr	mg kg^{-1} OM	18.5 (0.87)	11.6 (4.75)	9.0 (3.37)	6.2 (0.73)	27.7 (0.49)
	kg ha^{-1}	0.9 (0.04)	0.5 (0.22)	0.4 (0.16)	0.3 (0.03)	1.3 (0.02)
Cu	mg kg^{-1} OM	49.1 (0.13)	33.0 (3.41)	46.0 (9.71)	23.2 (1.29)	48.1 (12.5)
	kg ha^{-1}	2.3 (0.01)	1.5 (0.16)	2.1 (0.45)	1.1 (0.06)	2.2 (0.58)
Fe	mg kg^{-1} OM	16432 (1469)	3929 (1593)	9473 (3555)	2604 (398)	15596 (5553)
	kg ha^{-1}	766 (68.4)	183 (74.2)	441 (166)	121 (18.6)	727 (259)
K	mg kg^{-1} OM	1109 (204)	2008 (146)	855 (123)	1294 (83.2)	1645 (76.3)
	kg ha^{-1}	51.7 (9.53)	93.5 (6.82)	39.8 (5.72)	60.3 (3.88)	76.6 (3.55)
Mg	mg kg^{-1} OM	1719 (417)	1284 (321)	1251 (89.2)	783 (138)	1650 (157)
	kg ha^{-1}	80.1 (19.4)	59.8 (15.0)	58.3 (4.16)	36.5 (6.41)	76.9 (7.29)
Mn	mg kg^{-1} OM	179 (115)	667 (88.3)	43.3 (9.39)	1435 (80.1)	250 (128)
	kg ha^{-1}	8.3 (5.34)	31.1 (4.11)	2.0 (0.44)	66.9 (3.73)	11.7 (5.96)
Na	mg kg^{-1} OM	242 (70.4)	118 (14.1)	309 (45.5)	81.7 (7.39)	165 (63.2)
	kg ha^{-1}	11.3 (3.28)	5.5 (0.66)	14.4 (2.12)	3.8 (0.34)	7.7 (2.94)
Ni	mg kg^{-1} OM	18.6 (0.93)	13.6 (2.75)	18.4 (4.16)	9.1 (1.00)	18.4 (2.19)
	kg ha^{-1}	0.9 (0.04)	0.6 (0.13)	0.9 (0.19)	0.4 (0.05)	0.9 (0.10)
P	mg kg^{-1} OM	1666 (98.0)	1299 (85.0)	1266 (149)	1199 (143)	1515 (166)
	kg ha^{-1}	77.6 (4.57)	60.5 (3.96)	59.0 (6.92)	55.9 (6.65)	70.6 (7.74)
Pb	mg kg^{-1} OM	60.1 (7.1)	57.4 (5.26)	45.8 (11.4)	52.1 (4.89)	58.1 (3.20)
	kg ha^{-1}	2.8 (0.33)	2.7 (0.25)	2.1 (0.53)	2.4 (0.23)	2.7 (0.15)
S	mg kg^{-1} OM	2815 (309)	1856 (28.4)	2681 (150)	1921 (159)	2186 (181)
	kg ha^{-1}	131 (14.4)	86.5 (1.32)	125 (7.01)	89.5 (7.39)	102 (8.42)
Zn	mg kg^{-1} OM	54.8 (7.13)	144 (8.74)	32.5 (5.49)	132 (16.5)	86.7 (14.7)
	kg ha^{-1}	2.6 (0.33)	6.7 (0.41)	1.5 (0.26)	6.2 (0.77)	4.0 (0.69)
C	mg g^{-1} OM	523 (7.18)	527 (3.57)	541 (8.76)	562 (10.5)	517 (2.34)
	Mg ha^{-1}	23.0 (0.49)	23.2 (1.56)	24.9 (1.83)	23.6 (2.07)	19.7 (0.24)
N	mg g^{-1} OM	23.6 (1.34)	18.3 (0.12)	22.7 (0.60)	18.6 (1.41)	19.5 (0.77)
	kg ha^{-1}	1037 (51.2)	803 (53.9)	1045 (32.3)	776 (5.39)	746 (35.0)

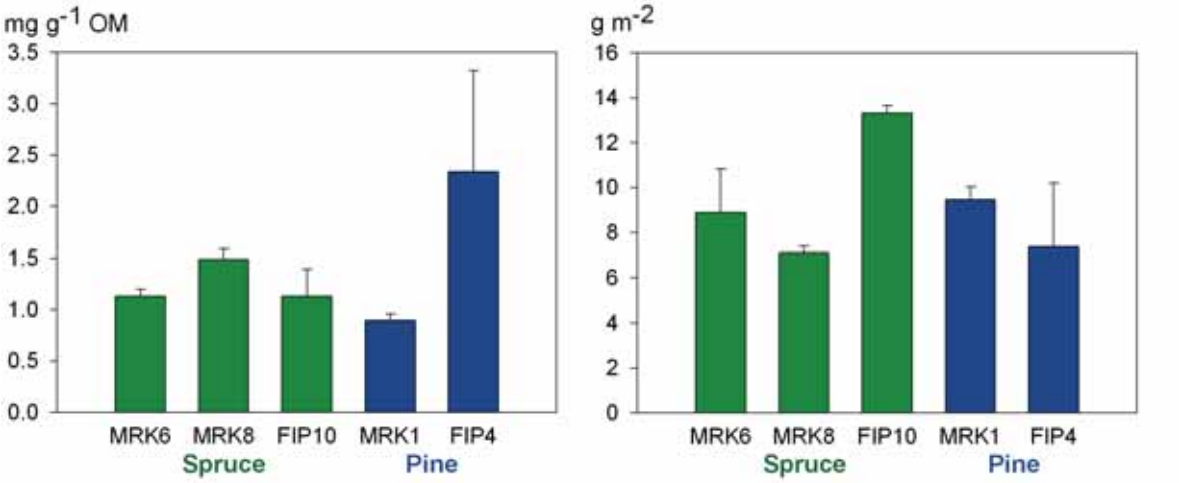

Figure 2. *Average and standard error of dissolved organic carbon (DOC) in the organic layer of study plots in Olkiluoto presented as concentrations and on area basis (n = 2).*

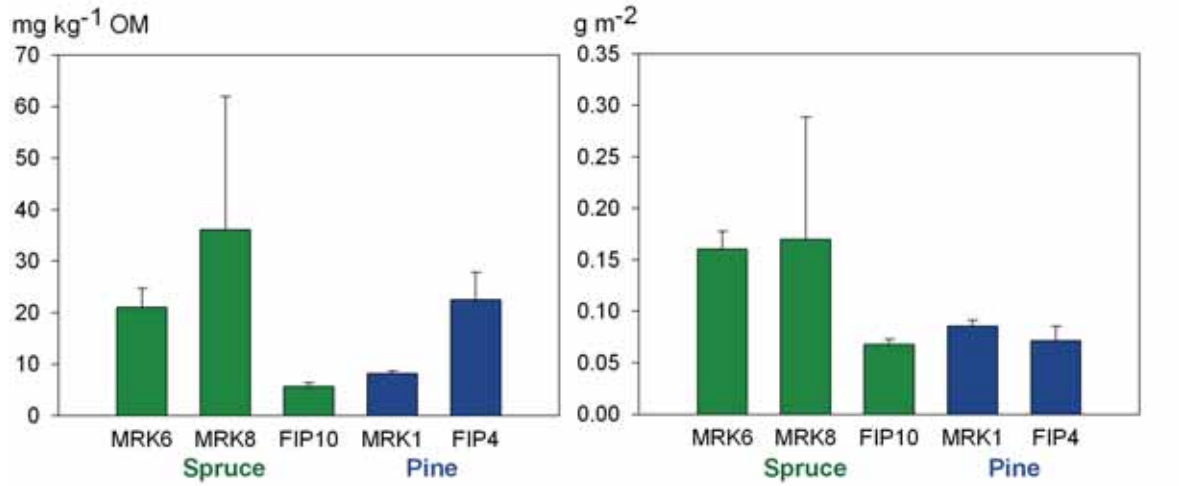

Figure 3. *Average and standard error of ammonium nitrogen (NH_4-N) in the organic layer of study plots in Olkiluoto presented as concentrations and on area basis (n = 2).*

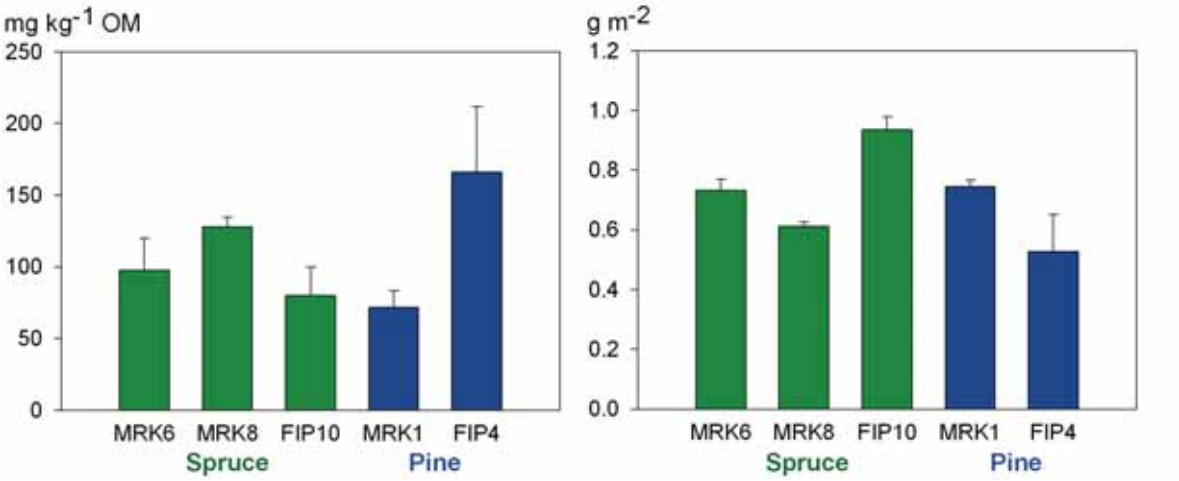

Figure 4. *Average and standard error of dissolved organic nitrogen (DON) in the organic layer of study plots in Olkiluoto presented as concentrations and on area basis (n = 2).*

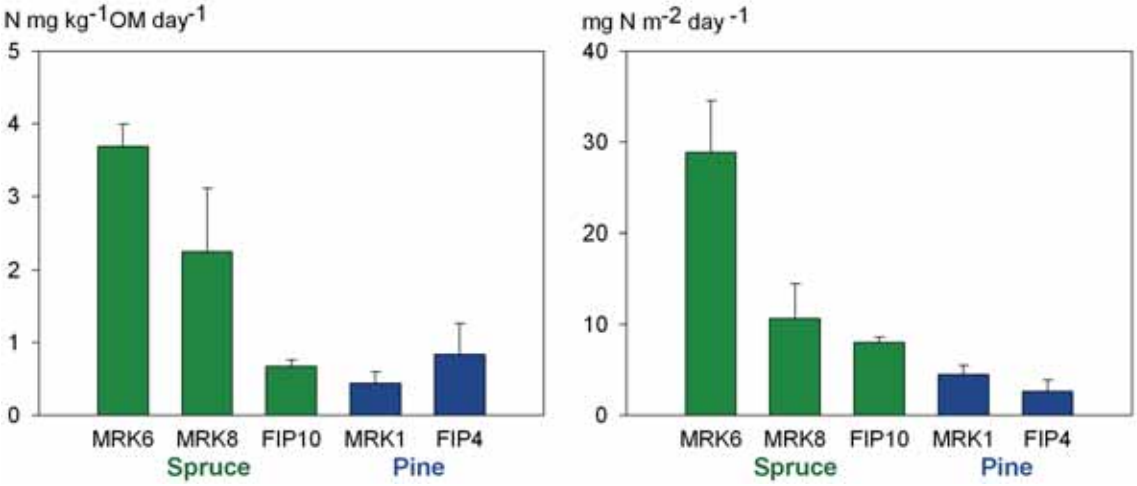

Figure 5. *Average and standard error of net N mineralisation per day in the organic layer of study plots in Olkiluoto presented as concentrations and on area basis (n = 2).*

0.28 mg kg^{-1} OM per day in one sample from MRK1 and the maximum 3.99 mg kg^{-1} OM per day in one sample from MRK6 (Appendix 2). The plots constituted three groups with respect to net N mineralisation after 31 days incubation: over 100 mg (MRK6), between 50 and 100 mg (MRK8), and under 50 mg kg^{-1} OM (FIP10, FIP4 and MRK1). Nitrification was not detected.

On an area basis, the average net N mineralisation of the organic layer per day was between 3 and 28 mg m^{-2} (Fig. 5). It varied from 1.4 mg in plot FIP4 to 34.5 mg m^{-2} in plot MRK6 (Appendix 2). The net N mineralisation was over three times higher under spruce than under pine. The average net N mineralisation per day was 15.8 mg m^{-2} under spruce, and 3.6 mg m^{-2} under pine.

3.4 Microbial biomass

Microbial biomass C (C_{mic}) in the organic layer varied from 7.2 to 13.2 mg g^{-1} OM, and was at the same level under spruce and under pine (10.6 mg g^{-1}, 10.7 mg g^{-1}, respectively). The average C_{mic} was lowest in plot FIP10 and highest in plot MRK8 (Fig. 6). On an area basis, the C_{mic} varied from 33.7 to 145.3 g m^{-2} and was higher under spruce than under pine (83.3 g m^{-2}, 74.7 g m^{-2}, respectively). C_{mic} was over 100 g m^{-2} in only one sample from plots FIP10 and MRK1, which considerably increased the average C_{mic} on these plots (Fig. 6, Appendix 2). The C_{mic} on an area basis correlated with the thickness of the organic layer (Spearman's $r = 0.842$, $P < 0.01$, $n = 10$).

Microbial biomass N (N_{mic}) varied from 0.5 mg in plot FIP10 to 1 mg g^{-1} OM in plots MRK8 and MRK6 (Appendix 2), and was at the same level under both tree species (spruce: 0.80 mg g^{-1} OM, pine: 0.82 mg g^{-1} OM). The average N_{mic} was the lowest in plot FIP10 and the highest in plot MRK8 (Fig. 7). There was high variation in the N_{mic} concentrations between the two samples from plot MRK6. On an area basis, N_{mic} varied from 2.7 to 8.4 g m^{-2}. The lowest average N_{mic} on an area basis was on plot FIP4 and the highest on

MRK1 (Fig. 7), reflecting the difference in the thickness of the organic layer (Spearman's correlation: $r = 0.794$, $P < 0.01$, $n = 10$).

Fungal biomass measured as ergosterol concentration varied from 306 to 868 µg g^{-1} OM, and the average ergosterol concentration was the lowest in plot FIP10 and the highest in plot FIP4 (Fig. 8). The concentration of fungal biomass was higher under pine (599 µg g^{-1} OM) than under spruce (405 µg g^{-1} OM). On an area basis, the amount of ergosterol varied between 2.0 and 4.5 g m^{-2} (Fig. 8), reflecting the difference in the thickness of the organic layer (Spearman's correlation: $r = 0.745$, $P < 0.05$, $n = 10$).

Principal component analysis (PCA) of the concentrations of dissolved N and C compounds and the microbial biomass grouped the plots according to the thickness of the organic layer (Fig. 9). Microbial biomass N, dissolved C and N compounds explained 58.2% of the variation between the plots along PC1 (principal component 1). Plots MRK1

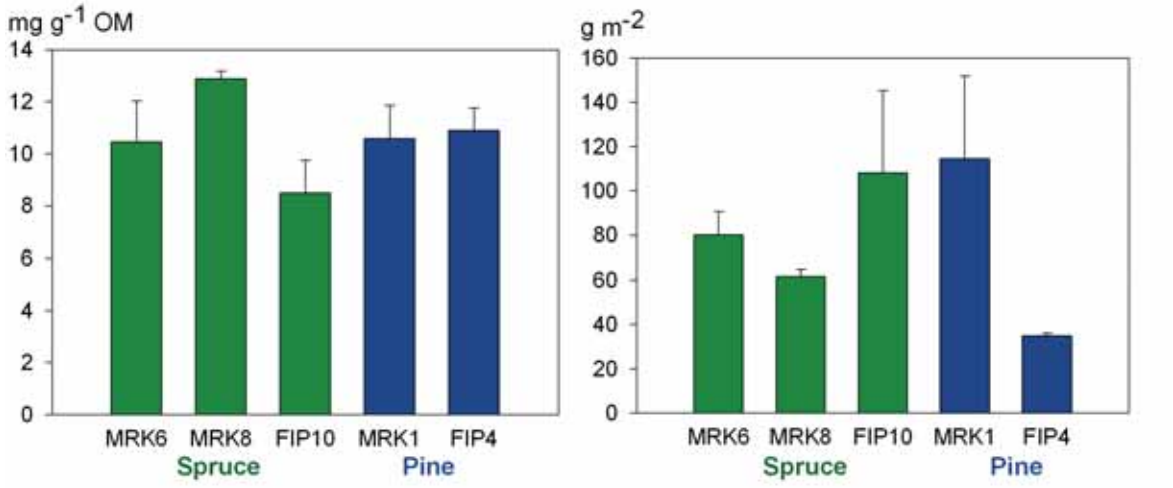

Figure 6. *Average and standard error of microbial biomass C (C_{mic}) in organic layer of study plots in Olkiluoto presented as concentrations and on area basis (n = 2).*

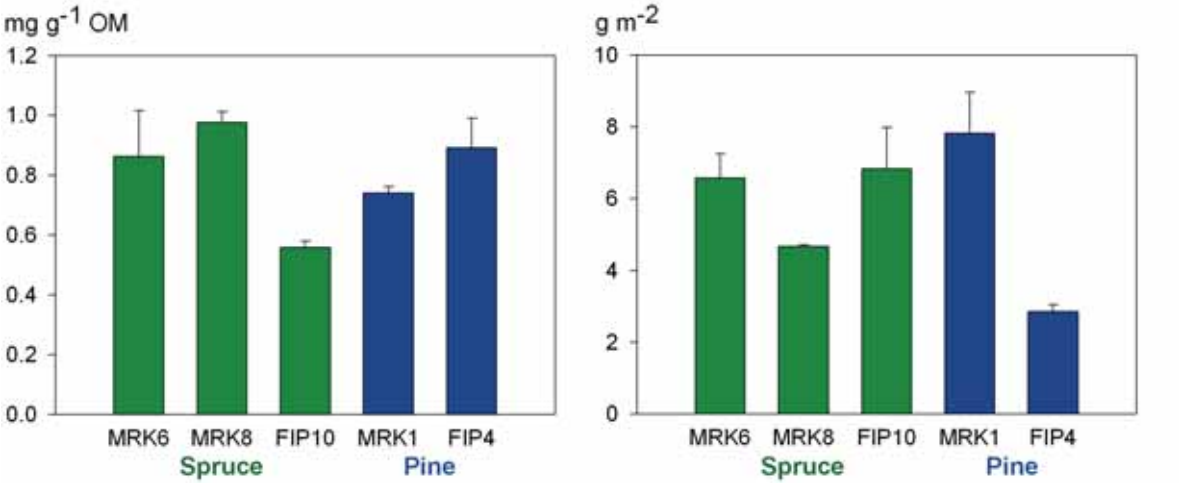

Figure 7. *Average and standard error of microbial biomass N (N_{mic}) in the organic layer of study plots in Olkiluoto presented as concentrations and on area basis (n = 2).*

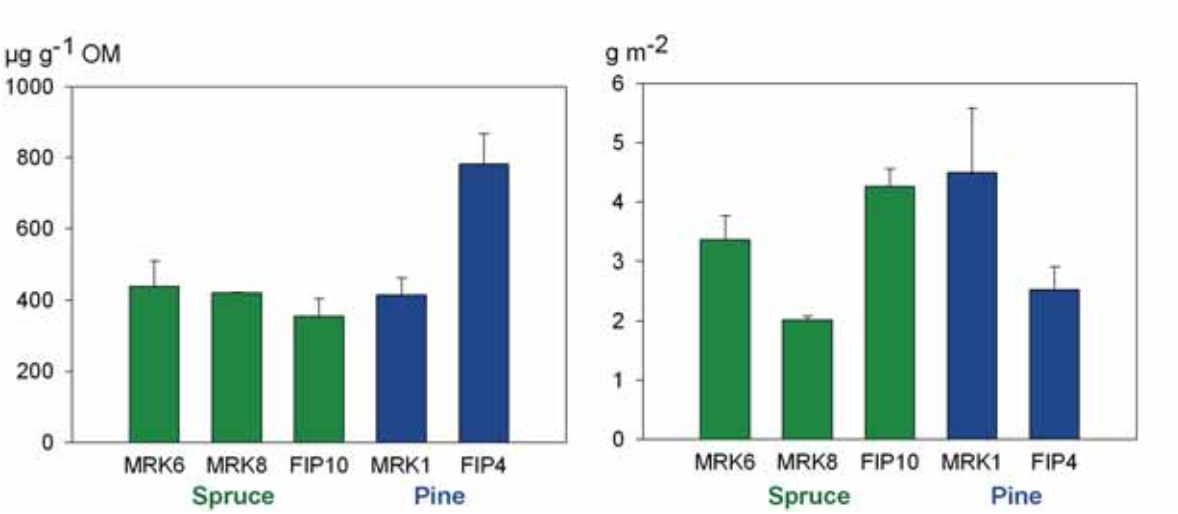

Figure 8. *Average and standard error of ergosterol in the organic layer of study plots in Olkiluoto presented as concentrations and on area basis (n = 2).*

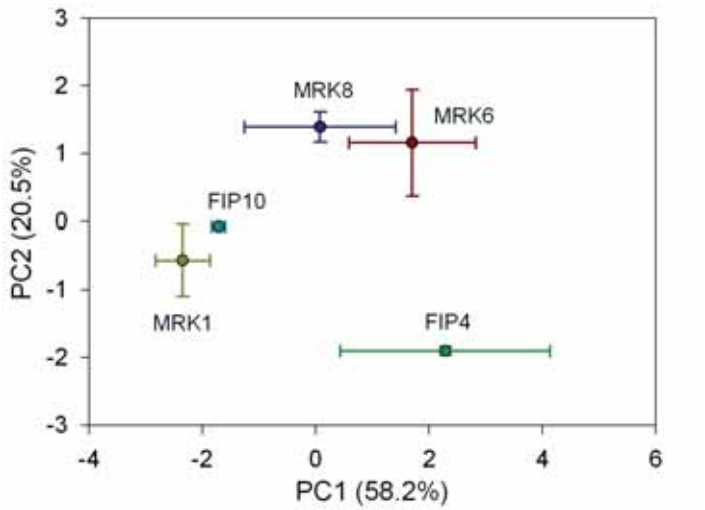

Figure 9. *Principal component analysis (PCA) ordination of dissolved C and N, net N mineralisation, ergosterol, microbial biomass N and C in organic layer showing separation of study plots along first two principal components (PC1 and PC2), average and standard error (n = 2).*

and FIP10, which had a thicker organic layer (88 mm and 90 mm, respectively), had very similar N transformation patterns. In these plots the variation between the samples was low. The rest of the plots were at the same level along PC1, but they had higher within-plot variation than the first group (MRK1 and FIP10). PC2 explained 20.5% of the variation caused mainly by the amount of net N mineralisation. This axis separated study plots MRK6 and MRK8 from study plot FIP4.

3.5 Fungal community structure

The DGGE profile of the plots is presented in Figure 10. The total number of ITS fragments in the DGGE profile varied between 9 and 14 (Fig. 11). This depicts the number of different ITS fragments (differences in length and/or base sequence) amplified from each sample.

The similarity indices of the replicate samples in each plot revealed that the DGGE profiles of the ITS fragments had the highest similarity in plot MRK6 ($C_j = 0.53$, $C_s = 0.70$) and the lowest in plot FIP4 ($C_j = 0.28$, $C_s = 0.43$) (Table 3). Furthermore, the similarity of the DGGE profiles of the ITS fragments were higher between the pine plots than between the spruce plots (Table 3). The same trend can be seen in nonmetric multidimensional scaling (MDS) of the DGGE profile of the ITS fragments in Figure 12, in which the DGGE profiles of the samples from MRK8 are the most similar and those from FIP4 the most dissimilar.

Direct sequencing of seven purified PCR samples (A–G) gave six sequences (B–G) (Fig. 10). Matches in BLASTN searches within the GenBank database were found for three sequences. Two sequenced bands revealed database sequences that had the highest similarity with unknown fungal sequences. The closest matches are presented in Table 4.

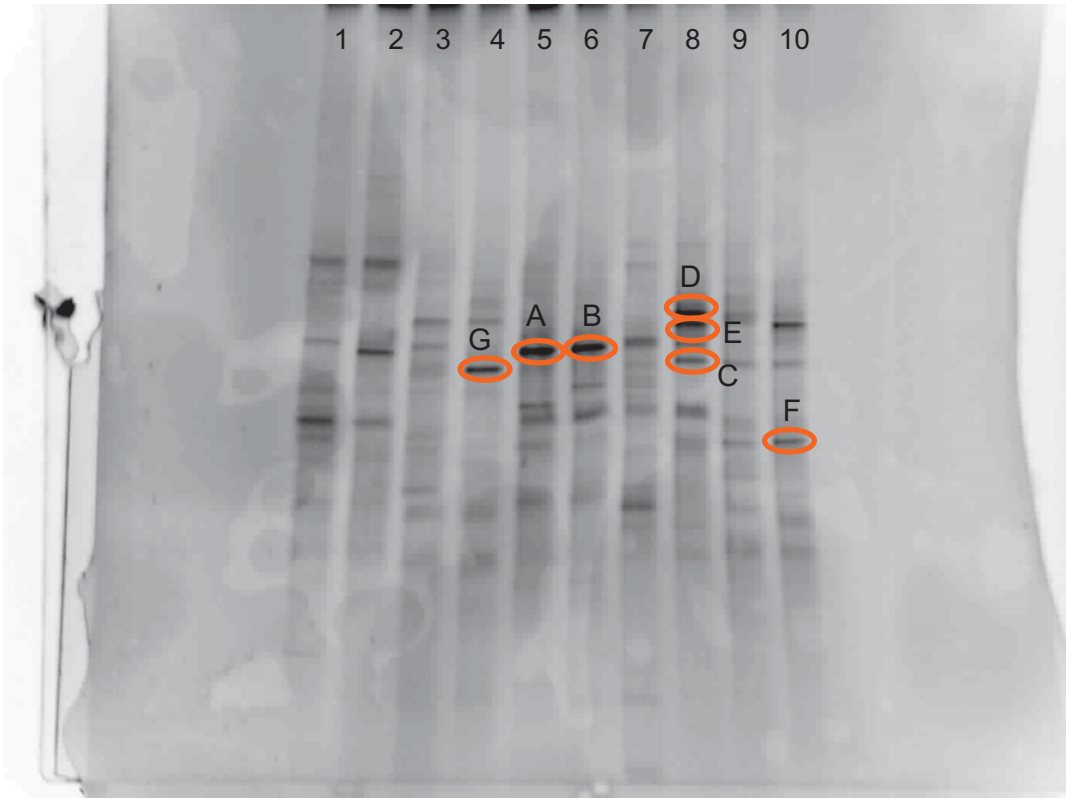

Figure 10. *Denaturing gradient gel electrophoresis (DGGE) of fungal ITS fragments amplified from organic layer of ten studied samples from Olkiluoto. Lane number corresponds to sampling point. Bands marked with letters (A-F) correspond to bands that were excised from DGGE and sequenced.*

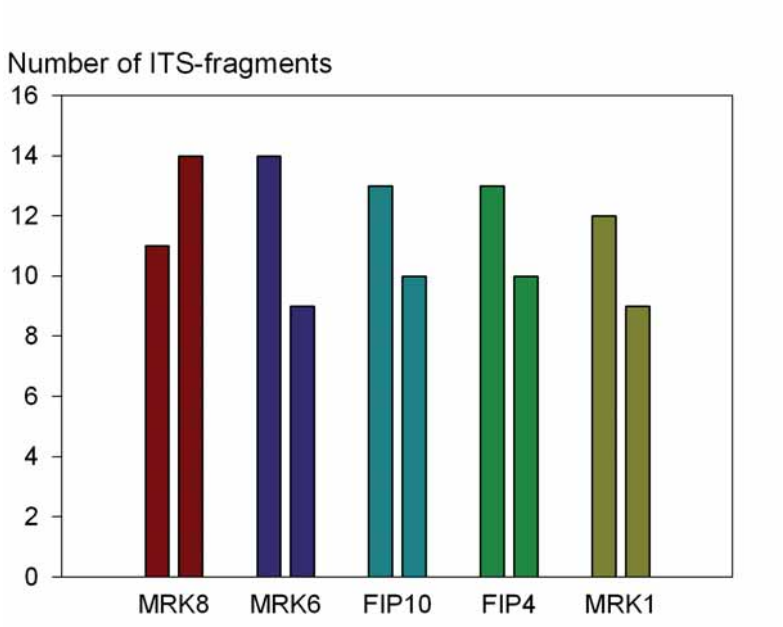

Figure 11. *Number of different fungal ITS-fragments, representing richness of taxa, separated from each sample in DGGE analysis.*

Table 3. *Number of shared ITS fragments (j) and similarity indices, Jaccard (C_j) and Sörensen (C_s) of DGGE profiles of the ITS fragments in the samples from the same plots (2 samples per plot) and from plots with the same tree species. The total number of ITS fragments was 38.*

	j	C_j	C_s
MRK8	8	0.47	0.64
MRK6	8	0.53	0.70
FIP10	6	0.4	0.57
FIP4	5	0.28	0.43
MRK1	7	0.44	0.61
Spruce plots	1	0.01	0.02
Pine plots	1	0.02	0.05

3.6 Community level physiological profile of the microbes, CLPP

The average colour development in the wells (AWCD) of the differently diluted samples during the incubation period is presented in Figure 13. The colour development depicts the utilization of available carbon sources in average.

The community level physiological profile (CLPP) of samples diluted 1:10 grouped the plots into three groups along the principal component one (PC1) (Fig. 14). FIP10 constituted one group, MRK1 and MRK6 a second group, and MRK8 and FIP4 a third group.

Along PC2 only MRK1 and MRK6 stood out from the rest. D-cellobiose, α-cyclodextrin, pyruvic acid methyl ester, L-asparagine, L-serine and putrescine were the substrates primarily responsible for the differences along PC1, which explained 29.7 % of the variation. Utilization of the first two substrates increased and utilization of the remaining substrates decreased to the right in the figure. The change in the utilization of β-methyl-D-glucoside was the main reason for the separation of study plot MRK1 and MRK6 from the others along PC2.

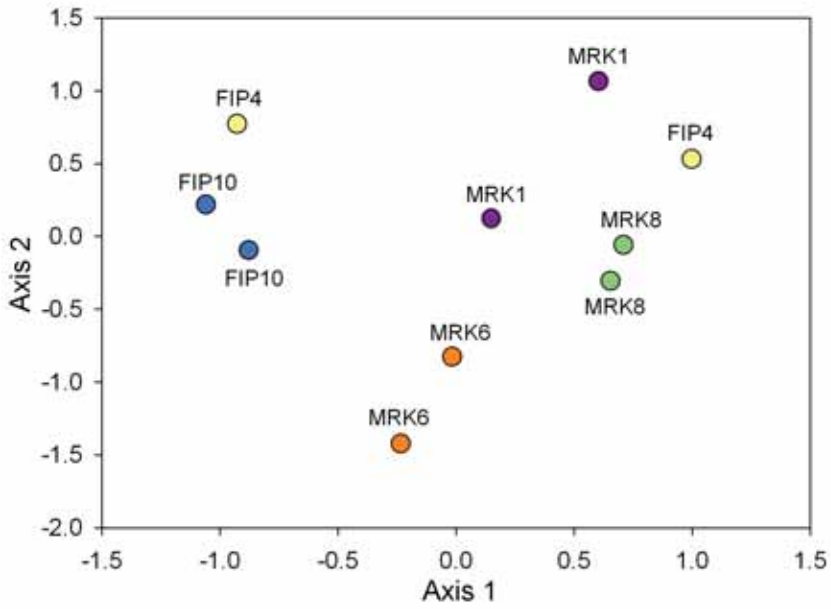

Figure 12. *Score plot of the MDS produced from DGGE data from ten organic soil samples of Olkiluoto showing the similarity (near) and dissimilarity (far) of samples. Number of ITS-fragments in analysis was 23.*

Table 4. *Identification of the sequences obtained from the organic layer of ten samples.*

Sequence	Length, base pair	Sequence similarity, %	Sequence affinity	Sample, plot
B	249	93% (232/249)	DQ309229.1, uncultured fungus	6, MRK1
C			No matches	8, FIP4
D	230	96% (187/193)	AY313293.1, *Rhodocollybia butyracea*	8, FIP4
E			No matches	8, FIP4
F	328	86% (160/185)	AY969697.1, uncultured basidiomycete	10, FIP10
G			No matches	4, MRK6

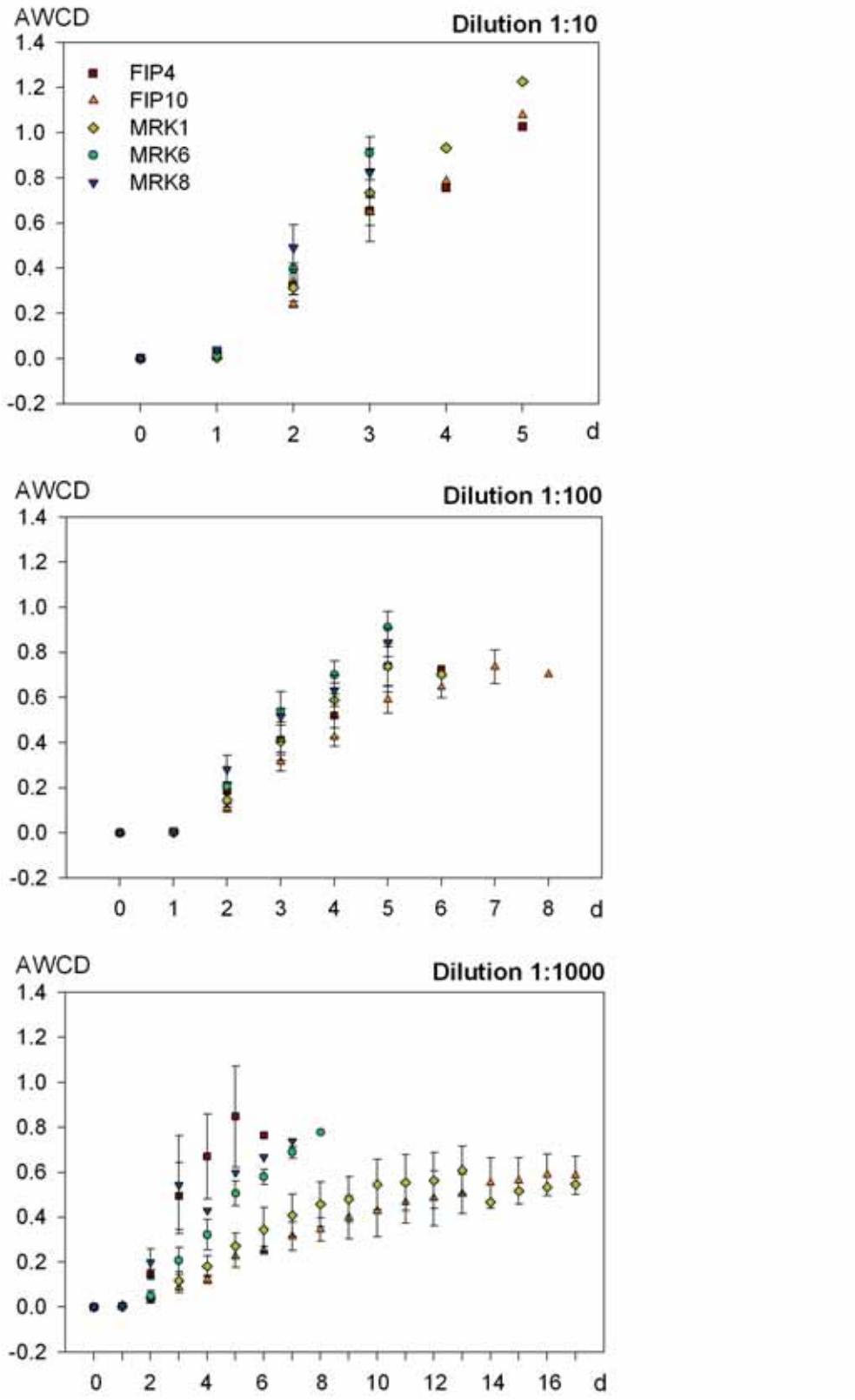

Figure 13. *Average well colour development (AWCD) as a function of time of organic layer samples in BIOLOG Ecoplates with standard errors of the mean from two replicate samples per plot at different dilution rates. The difference on color development intensity between different dilutions is shown here.*

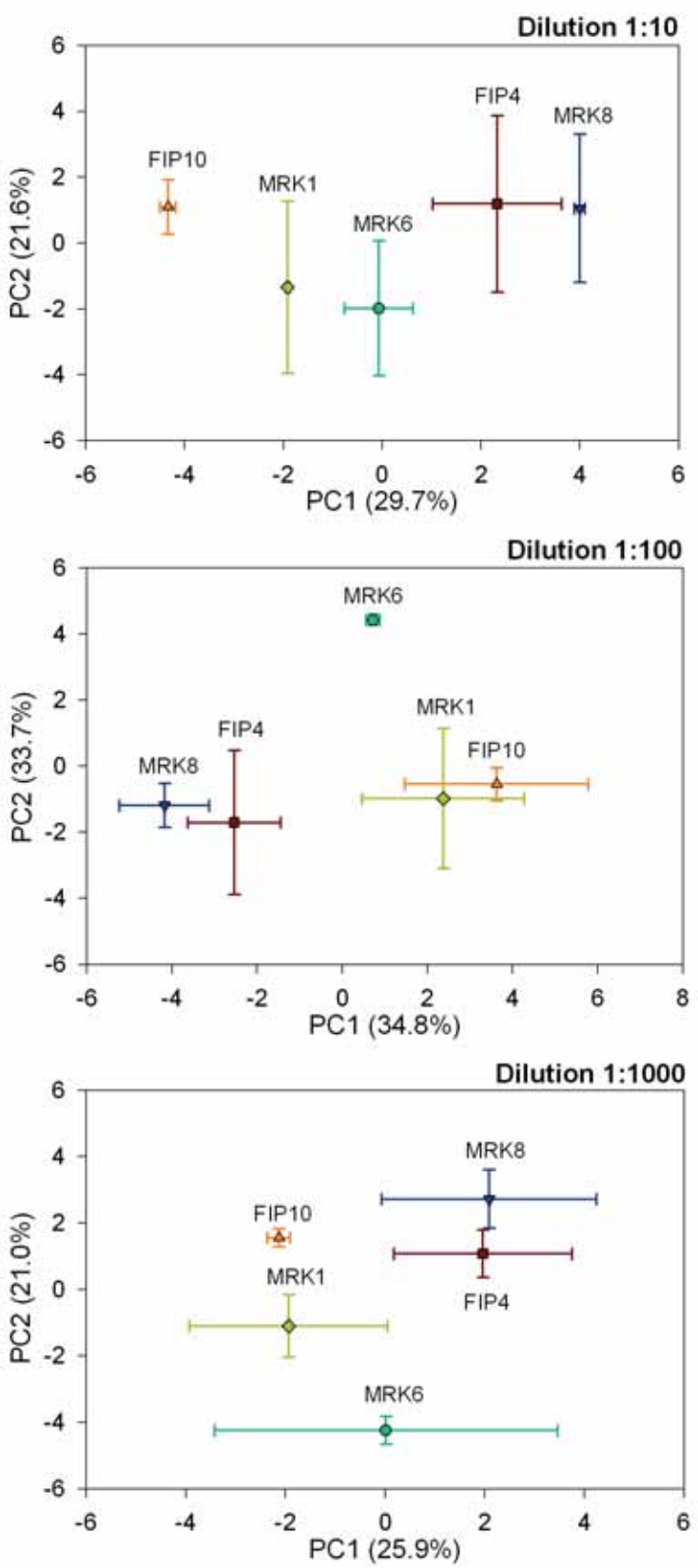

Figure 14. *PCA ordination of community level physiological profile (CLPP) for different dilutions showing the separation of study plots along the first two principal components (PC1 and PC2) using AWCD data of BIOLOG Ecoplates at AWCD = 0.6 reading point (n = 2).*

Sample dilutions 1:100 and 1:1000 CLPP resulted in three groups (Fig. 14). FIP4 and MRK8 constituted one group, MRK1 and FIP10 a second group and MRK6 a third group. The groups were even clearer with dilution 1:100 than with dilution 1:1000, and the degree of explanation of the variation was higher with dilution 1:100 than with dilution 1:1000 (Fig. 14). However, the individual substrates primarily responsible for the differences along PC1 and PC2 differed between the dilutions (Table 5).

Overall, the number of utilized substrates increased during incubation (Table 6), whereas with dilution 1:10 the number of utilized C sources decreased during incubation in MRK6, FIP10 and MRK1. The Shannon diversity index (H), which indicates the richness of utilized carbon sources in CLPP analysis, seemed to decrease with increasing dilution at the first reading (AWCD = 0.2), but this trend disappeared in the later readings (Table 6). Furthermore, with one dilution H mainly increased when AWCD increased (Table 6). Evenness (E), which indicates the relative abundance of utilized carbon sources in CLPP analysis, seemed to increase during incubation in all dilutions (Table 6). The evenness was slightly lower in dilution 1:1000 at AWCD 0.2 and 0.6 than in the others.

Table 5. *The individual carbon substrates primarily responsible for the changes along PC1 and PC2 in dilution 1:100 and in dilution 1:1000.*

	PC1	PC2
Dilution 1:100	Tween 40	Pyruvic acid methyl ester
	Tween 80	i-Erythritol
	D-Mannitol	L-Theonine
	D-Gulosaminic acid	Glycyl-L-glutamic acid
	Glucose-1-phosphate	
	L-Arginine	
	L-Phenylalanine	
Dilution 1:1000	D-Cellobiose	Pyruvic acid methyl ester
	D-Galactonic acid γ-lactone	Tween 40
	α-Keto butyric acid	D-Mannitol
	L-Theonine	

Table 6. *Number of utilized carbon sources (S, n of available carbon sources = 31), Shannon diversity (H) and evenness (E) of Ecoplates (Biolog) in the plots. Indices were calculated at three different reading points according to the average well colour development (AWCD) in the plate. Values are mean and SE in parentheses.*

	S			H			E		
AWCD	0.2	0.4	0.6	0.2	0.4	0.6	0.2	0.4	0.6
Dilution 1:10									
MRK6		29 (0)	28 (2.0)		3.02 (0.05)	3.16 (0.05)		0.90 (0.01)	0.95 (0.01)
MRK8		29	29.5 (0.5)		3.10	3.17 (0.07)		0.92	0.94 (0.02)
FIP10	27.5 (0.5)		26.5 (2.5)	2.90 (0.00)		3.04 (0.05)	0.88 (0.00)		0.93 (0.01)
FIP4	27.5 (2.5)	28	29 (1.0)	3.04 (0.03)	3.03	3.17(0.03)	0.92 (0.01)	0.91	0.94 (0.00)
MRK1	29	28	26.5 (0.5)	2.98	2.95	3.12 (0.01)	0.88	0.88	0.95 (0.01)
Dilution 1:100									
MRK6	24.5 (1.5)		27 (2.0)	2.82 (0.02)		3.04 (0.01)	0.88 (0.01)		0.92 (0.02)
MRK8	28	28 (0)	29.5 (0.5)	3.08	3.04 (0.10)	3.18 (0.04)	0.92	0.91 (0.03)	0.94 (0.01)
FIP10	24.5 (0.5)	27.5 (0.5)	29.5 (0.5)	2.75 (0.02)	2.89 (0.02)	3.10 (0.07)	0.86 (0.00)	0.87 (0.00)	0.92 (0.02)
FIP4	27.5 (0.5)	28.5 (1.5)	29 (1.0)	3.03 (0.04)	3.13 (0.04)	3.22 (0.04)	0.91 (0.01)	0.94 (0.00)	0.96 (0.00)
MRK1	26.5 (1.5)	27.5 (0.5)	29 (1.0)	2.85 (0.12)	3.00 (0.06)	3.17 (0.00)	0.87 (0.02)	0.90 (0.02)	0.94 (0.01)
Dilution 1:1000									
MRK6	24.5 (1.5)	26.5 (2.5)	27.5 (0.5)	2.72 (0.17)	2.85 (0.19)	2.94 (0.12)	0.85 (0.04)	0.87 (0.03)	0.89 (0.03)
MRK8	27.5 (0.5)	30	29 (1.0)	2.85 (0.13)	3.20	3.14 (0.12)	0.86 (0.03)	0.94	0.93 (0.02)
FIP10	29 (1)	30 (1)	30.5(0.5)	2.85 (0.19)	3.09 (0.04)	3.16 (0.07)	0.85 (0.05)	0.91 (0.00)	0.92 (0.02)
FIP4	28.5 (0.5)	29	29.5 (0.5)	2.93 (0.02)	3.09	3.12 (0.07)	0.87 (0.00)	0.92	0.92 (0.02)
MRK1	28 (0)	28.5 (0.5)	29 (0)	2.86 (0.02)	3.03 (0.03)	3.12 (0.08)	0.86(0.01)	0.90 (0.01)	0.93 (0.02)

4 DISCUSSION

4.1 Nitrogen transformations and microbial community structure

There was high variation in the total nutrient concentrations between the samples (Table 2). In most cases, however, the total nutrient concentrations were slightly lower than those found on the FEH plots in Olkiluoto (Tamminen et al. 2007). Especially in plots with the thickest organic layer the standard error of the mean for many nutrients was over 10 % of the average value, thus confirming the high within-plot variation in the total nutrient concentrations in the plots. The Mn concentration in plot FIP4 was twice that reported by Tamminen et al. (2007) for OMT-type forest plots on Olkiluoto (see Table 1 for explanation of forest site types).

Merilä et al. (2002) studied soil nitrogen transformation in 95-year-old and 130-year-old spruce stands on Raippaluoto, another island in the Gulf of Bothnia to the north of Olkiluoto. The concentrations of NH_4^+-N, NO_3^--N and DON in September 1997 were 33 mg kg^{-1} OM, 0 mg kg^{-1} OM, 210 mg kg^{-1} OM, respectively, in the 95-year-old stand, and 15 mg kg^{-1} OM, 0 mg kg^{-1} OM, 161 mg kg^{-1} OM, respectively, in the 130-year-old stand. Our results were at approximately the same level, but it is known that the concentrations of dissolved N compounds can vary considerably within the season and between years (Merilä et al. 2002, Potila & Sarjala 2004), depending on climatic factors such as temperature and precipitation.

In Raippaluoto the net N mineralisation rate in the laboratory incubation was ca. 1 mg kg^{-1} OM day^{-1} in September in the 130-year-old spruce stand, and ca. 4 mg kg^{-1} OM day^{-1} in the 95-year-old spruce stand (Merilä et al. 2002). They did not find any net nitrification. In our study, the net N mineralisation was 2.1 mg kg^{-1} OM day^{-1} in the spruce stands, and higher in the younger stands. In pine stands the net N mineralisation was 0.76 mg kg^{-1} OM day^{-1}. These results are consistent with those of Priha & Smolander (1999), who reported lower net N mineralisation under pine than under spruce in 60-year-old OMT-type stands in Punkaharju, eastern Finland.

Merilä et al. (2002) reported an N_{mic} of about 1100 mg kg^{-1} OM in a 95-year-old spruce stand and 820 mg kg^{-1} OM in a 130-year-old spruce stand. These values are at the same level as those measured in the organic layer samples in Olkiluoto. The N_{mic} concentration was lower in plot FIP10 than in the other plots. The low C/N ratio in this plot (Table 2) may indicate that microbial growth is limited by factors other than nitrogen, e.g. by recalcitrant C sources.

According to Möttönen et al. (1999), the humus ergosterol concentration varied from 64 to 824 mg kg^{-1} OM in a 150- to 200-year-old *Vaccinium-Myrtillus* type Scots pine stand in eastern Finland. In the pine forest soils in Olkiluoto the ergosterol concentration was 599 µg g^{-1} OM in the organic layer. Möttönen et al. (1999) postulated that the spatial pattern of ergosterol was most strongly related to pH, organic layer thickness and the total C concentration. A dependence of the ergosterol concentration and the organic layer thickness was also found in our study.

Diversity parameters, evenness (E) and the Shannon – Wiener (H) diversity index describe the number of utilized carbon sources and the intensity in which they are utilized. In the present study, according to the Shannon diversity index (H) increased dilution of the sample resulted in the utilization of fewer C sources at the beginning of the incubation. Furthermore, the number of utilized C sources and intensity of utilization increased concurrently during incubation with increasing size of the bacterial population in the wells.

Diversity at the functional level rather than at the taxonomic level is crucial for the long term stability of an ecosystem (Insam & Goberna 2004). The transformed parameters, i.e. absorbances divided by AWCD, provide qualitative information about CLPP in soils. The PCA of CLPP showed differences in functional diversity between the plots. Because the density of the bacterial population in the inoculum affects the information given by the CLPP, different rates of sample dilution were necessary to clarify the results.

According to MDS, the fungal community was the most similar between the replicate samples in MRK8, whereas the similarity index gave the most similar fungal community in the replicate samples of plot MRK6. The difference in the statistical evaluation of the results by MDS or similarity index was caused by the number of species included in the statistical analysis. In the case of the similarity index all 38 different ITS fragments were taken into account in the calculations, whereas in MDS the ITS fragments with less than two observations were excluded (n = 23). Thus MDS emphasized the similarity or dissimilarity of common ITS fragments.

Three matches of ITS fragment sequences were found in the GenBank database. Sequence D matched with species *Rhodocollybia butyracea*. This sequence was extracted from OMT-type pine stand. *Rhodocollybia butyracea* (valkoviirujuurekas in Finnish) is a common saprotrophic species in Finland in coniferous forest, especially in spruce stands. Matches were also found with two unknown fungi. Nucleotide databases accept scientific names only for those fungus species which have been sequenced from sporocarps, and our current understanding of fungal biodiversity in soil is limited (Anderson & Cairney 2004). Because all fungal species have not yet been sequenced, the range of species available in the databases is relatively limited. O'Brien et al. (2005) estimated that global soil fungal species richness ranges from 3.5 to 5.1 million. Their estimate was based on 412 sequence types from 863 fungal ITS sequences extracted from different soil layers. The sequences without matches are due to the lack of corresponding sequences in the databases, which means that these fungal strains have not been sequenced or submitted to the databases before by any other laboratory.

Only a few studies have been carried out on microbial communities in land up-lift coasts (e.g. Aikio 2000, and Merilä 2002). These studies concerned different successional stages of the forest ecosystems that appear on land up-lift coasts. The developing forest vegetation depends on the physiological and chemical properties of the soil rising up from the sea, and reflects the microbial community. In both studies (Aikio 2000, Merilä 2002) they found that plant and soil microbes became increasingly N limited during forest succession. Microbial biomass and activity increased (Aikio 2000) or were relatively stable

(Merilä 2002) along the successional forest gradient. Merilä (2002) observed clear differences in the structure of the microbial community along a successional transect.

4.2 Application of the techniques

4.2.1 Molecular techniques

Studying soil fungal diversity by direct nucleic acid extraction has some methodological and data analysis limitations. One problem is the quality and purity of the extracted nucleic acid pool. The humic acids and humic substances that are co-extracted together with nucleic acids from soil can inhibit DNA-modifying enzymes. Furthermore, estimates of soil fungal diversity can be markedly affected by the extraction technique used (Anderson & Cairney 2004.) Even the sample size can influence the obtained diversity. It has been shown that fungal community structure changes if sample size used for DNA extraction is less than 1 g (Ranjard et al. 2003). A further complicating factor is the potential presence of both spores and mycelium in an individual soil sample, both of which will probably be co-extracted during the DNA extraction process. Because the longevity of fungal spores in soils is unclear, this must be kept in mind when considering soil mycelial communities (Anderson & Cairney 2004.)

Because the DNA pool extracted from soil constitutes DNA from a diverse range of eukaryotic and prokaryotic organisms, finding suitable PCR primers to amplify all members of the fungal community without bias has been one of the major limitations in investigating fungal diversity in soils (Anderson et al. 2003). Numerous PCR primers for amplifying fungal rDNA (ribosomal DNA) from different taxonomic groups have been developed, although few of them were explicitly designed for use with extracts from mixed communities. According to Anderson et al. (2003), both 18S rDNA and ITS primers are useful in assessing the biodiversity of fungal communities in complex environmental samples, but both have their limitations. Further, PCR amplification might promote the amplification of abundant species, thus missing some less common species.

DGGE has been one of the most widely used community fingerprinting techniques in soil microbial ecology, hence the potential and pitfalls of the techniques have been well documented by Muyzer and Smalla (1998). One of the main advantages of gel-based community profiling techniques is the ability to excise and sequence bands of interest. However, short fragments of DNA limit the taxonomic information that can be obtained by sequencing excised bands. Furthermore, the less dominant members of a community may not be detected, and a single band in a gel can comprise more than one sequence type (Muyzer & Smalla 1998, Anderson & Cairney 2004).

Common ordination methods include non-metric multidimensional scaling (MDS), principal component analysis (PCA), correspondence analysis (CA), canonical variate analysis (CVA) and canonical correspondence analysis (CCA). The advantage of MDS is that it represents the objects in two or three dimensions, with dissimilar objects far apart and similar objects close to one other in the ordination space. The most often used method

is PCA. However, PCA is probably not the most suitable statistical approach for analyzing DGGE patterns, because it assumes that biological populations have linear response curve along the axis of ecological variation. Correspondence analysis has been found to be well suited for populations with a unimodal distribution along environmental gradients, and thus it may be applied to any data table that is dimensionally homogeneous (Fromin et al. 2002).

4.2.2 Community level physiological profile

Ecoplate provides a fast screening method to detect differences among samples. Because of the growing conditions and environment on the plate, however, it does not assess the real soil community. It assesses that fraction of the soil microbial community which can grow rapidly in liquid media in an Ecoplate environment. Thus, due to its limitations it should not be seen as a stand-alone method. The utilization of a substrate by the microbial community in the Ecoplate might not necessarily couple with changes in the availability of the particular substrate in the soil, and the changes in CLPPs can reflect either changes in community composition or adaptation of the microbial community (Insam & Goberna 2004).

4.2.3 Fungal biomass

Ergosterol has been widely used as an estimate of vital fungal biomass. However, the existence of exocellular ergosterol and/or ergosterol in recently dead fungal biomass in soils may affect the results of ergosterol analysis (Zhao et al. 2005). Variation of ergosterol concentrations within and between fungus species has been reported by Martin et al. (1990) and Ekblad (1995). However, diversity of the fungus species present in the soil dampens the possible effect of this variation.

4.2.4 Mineralisation

Net-N mineralisation rate, which is determined by excluding the N uptake by the roots, can under- or overestimate the real mineralization in situ. This depends on the competition rate for C and N between soil microorganism and plant/mycorrhizal associations, and the effects of root exudates on the microbial community (Persson et al. 2000).

5 CONCLUSIONS

This study provides descriptive data about the microbial biomass, community structure and activity in the organic layer of forest soils on Olkiluoto in September 2006. In this study the variation in the level of N mineralisation, dissolved N compounds, fungal biomass and microbial community structure in the plots were within the normal range of other published data for similar forest types in Finland.

Only a few studies have been earlier carried out on microbial communities in land up-lift coasts (Aikio 2000, Merilä 2002), and the results of our study did not differ to any marked extent. Our aim was not, however, to describe changes in microbial community along successional stages of the up-lift coastal gradient in Olkiluoto, and the results cannot be compared as such with the results of Aikio (2000) or Merilä (2002).

The characteristics of microbial communities are important from the point of view of the nutrient fluxes in the soil. In the present study, N mineralisation rate was higher under spruce than pine, whereas more fungal biomass was found in the soil under pine suggesting that the soil nutrient fluxes in the pine plots were more strongly fungus-driven than the spruce plots.

In general, microbial community structure and activity vary spatially and temporally, and they have a non-random spatial distribution (Sylvia et al. 1999). The functioning and structure of the soil biota in this case study was characterized by large variation within and between the plots, and this should be taken into account in future studies.

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Appendix 1. *Total elemental concentrations in the the organic layer of each sampling point.*

Element	Sample nr. / Plot	1 MRK8	2 MRK8	3 MRK6	4 MRK6	5 MRK1	6 MRK1	7 FIP4	8 FIP4	9 FIP10	10 FIP10
Al	mg kg OM^{-1}	1746	4556	7263	8333	12409	7454	2122	2920	3761	9194
	kg ha^{-1}	81	212	338	388	578	347	99	136	175	428
B	mg kg OM^{-1}	9.88	9.63	4.89	5.57	5.73	5.58	5.98	8.62	6.43	7.30
	kg ha^{-1}	0.46	0.45	0.23	0.26	0.27	0.27	0.28	0.40	0.30	0.34
Ca	mg kg OM^{-1}	7838	6802	3881	6284	5378	4154	4640	6460	6858	4045
	kg ha^{-1}	365	317	181	293	251	194	216	301	320	188
Cd	mg kg OM^{-1}	0.94	1.21	1.21	0.88	1.04	0.66	1.06	0.83	0.53	0.78
	kg ha^{-1}	0.04	0.06	0.06	0.04	0.05	0.03	0.05	0.04	0.02	0.04
Cr	mg kg OM^{-1}	6.82	16.3	17.6	19.4	28.2	27.2	5.50	6.96	5.67	12.4
	kg ha^{-1}	0.32	0.76	0.82	0.90	1.31	1.27	0.26	0.32	0.26	0.58
Cu	mg kg OM^{-1}	29.6	36.4	49.3	49.0	60.6	35.6	21.9	24.5	36.3	55.7
	kg ha^{-1}	1.38	1.70	2.30	2.28	2.83	1.66	1.02	1.14	1.69	2.60
Fe	mg kg OM^{-1}	2335	5522	14964	17901	21148	10043	2206	3003	5918	13027
	kg ha^{-1}	109	257	697	834	985	468	103	140	276	607
K	mg kg OM^{-1}	1861	2154	13134	905	1569	1721	1211	1377	732	978
	kg ha^{-1}	86.7	100	61.2	42.2	73.1	80.2	56.4	64.2	34.1	45.5
Mg	mg kg OM^{-1}	963	1606	1302	2136	1807	1494	645	920	1162	1340
	kg ha^{-1}	44.9	74.8	60.6	99.5	84.2	69.6	30.1	42.9	54.1	62.4
Mn	mg kg OM^{-1}	755	578	293	64.1	123	378	1355	1515	34.0	52.7
	kg ha^{-1}	35.2	26.9	13.7	2.99	5.71	17.6	63.1	70.6	1.58	2.46
Na	mg kg OM^{-1}	104	132	172	312	228	102	74.3	89.1	354	263
	kg ha^{-1}	4.83	6.14	7.99	14.6	10.6	4.75	3.46	4.15	16.5	12.3
Ni	mg kg OM^{-1}	10.8	16.3	17.6	19.5	20.6	16.2	8.13	10.1	14.3	22.6
	kg ha^{-1}	0.50	0.76	0.82	0.91	0.96	0.76	0.38	0.47	0.66	1.05

Appendix 1. *Total elemental concentrations in the the organic layer of each sampling point (cont´d).*

Element	Sample nr. / Plot	1 MRK8	2 MRK8	3 MRK6	4 MRK6	5 MRK1	6 MRK1	7 FIP4	8 FIP4	9 FIP10	10 FIP10
P	mg kg OM^{-1}	1214	1384	1764	1568	1681	1349	1056	1342	1117	1414
	kg ha^{-1}	56.6	64.5	82.2	73.0	78.3	62.9	49.2	62.5	52.1	65.9
Pb	mg kg OM^{-1}	52.1	62.7	67.2	53.0	54.9	61.3	47.2	57.0	34.4	57.2
	kg ha^{-1}	2.43	2.92	3.13	2.47	2.56	2.86	2.20	2.66	1.60	2.66
S	mg kg OM^{-1}	1884	1828	2506	3123	2367	2006	1763	2080	2832	2531
	kg ha^{-1}	87.8	85.2	117	146	110	93.4	82.1	96.9	132	118
Zn	mg kg OM^{-1}	135	153	61.9	47.7	72.0	101	149	116	27.0	38.0
	kg ha^{-1}	6.30	7.12	2.88	2.22	3.35	4.73	6.93	5.39	1.26	1.77
C	mg g OM^{-1}	531	464	504	483	429	418	551	462	575	496
	Mg ha^{-1}	24.7	21.6	23.5	22.5	20.0	19.5	25.7	21.5	26.8	23.1
N	mg g OM^{-1}	18.4	16.1	21.2	23.4	16.8	15.3	16.5	16.8	23.1	21.7
	kg ha^{-1}	856	749	986	1088	781	711	770	781	1077	1013

Appendix 2. *Thickness of the organic layer, pH, organic matter content (OM), C/N ratio, concentrations and amounts of ammonium and total N and dissolved N and C compounds (DON, DOC), microbial biomass C and N (C_{mic}, N_{mic}), ergosterol and net N mineralisation in the organic layer of each sampling point.*

	Samble nr. Plot	1 MRK8	2 MRK8	3 MRK6	4 MRK6	5 MRK1	6 MRK1	7 FIP4	8 FIP4	9 FIP10	10 FIP10
Thickness	cm	39.6	42.2	41.5	57.8	97.5	81.8	31.2	27.8	107	69.4
pH	H_2O	4.20	4.20	3.84	4.17	4.27	3.88	3.99	4.08	3.93	3.95
	CaCl	3.71	3.66	3.37	3.74	3.77	3.31	3.40	3.58	3.45	3.48
OM	%	86.5	76.6	82.2	81.0	71.4	70.3	83.4	72.6	90.4	80.6
C to N ratio		28.9	28.8	23.8	20.7	25.6	27.4	33.4	27.6	24.9	22.8
NH_4-N	mg g^{-1} OM	0.062	0.010	0.025	0.017	0.008	0.009	0.017	0.028	0.005	0.006
	g m^{-2}	0.289	0.051	0.143	0.178	0.091	0.080	0.058	0.086	0.073	0.063
TOT-N	mg g^{-1} OM	0.197	0.132	0.145	0.093	0.068	0.092	0.138	0.240	0.065	0.106
	g m^{-2}	0.916	0.648	0.839	0.947	0.814	0.848	0.463	0.737	0.964	1.041
DON	mg g^{-1} OM	0.135	0.122	0.120	0.075	0.060	0.083	0.121	0.212	0.060	0.010
	g m^{-2}	0.627	0.598	0.696	0.769	0.723	0.767	0.405	0.651	0.891	0.978
DOC	mg g^{-1} OM	1.59	1.39	1.20	1.06	0.832	0.960	1.35	3.32	0.873	1.39
	g m^{-2}	7.41	6.81	6.97	10.83	10.04	8.86	4.54	10.22	13.01	13.65
C_{mic}	mg g^{-1} OM	12.59	13.18	12.01	8.89	11.93	9.25	10.03	11.75	9.75	7.24
	g m^{-2}	58.7	64.8	69.7	90.7	143.8	85.3	33.7	36.1	145.3	70.9
N_{mic}	mg g^{-1} OM	1.013	0.938	1.016	0.711	0.695	0.786	0.793	0.990	0.536	0.580
	g m^{-2}	4.72	4.61	5.89	7.25	8.38	7.25	2.66	3.04	7.98	5.68
ergosterol	µg g^{-1} OM	421	422	509	369	463	369	868	696	306	405
	g m^{-2}	1.96	2.08	2.95	3.76	5.58	3.40	2.91	2.14	4.56	3.97
Net N	mg kg^{-1} OM d^{-1}	3.11	1.39	3.99	3.39	0.28	0.60	0.42	1.26	0.58	0.76
mineralisation	mg m^{-2} d^{-1}	14.5	6.84	23.1	34.5	3.43	5.54	1.40	3.88	8.60	7.46