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Potential impacts of clear-felling on microbial activities in boreal humus and mineral soil layers

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Changes affecting the microbial activity in boreal coniferous forest humus and mineral soil layers after clear-felling were investigated by studying exoenzyme activities, microbial colony counts and community level physiological profiles, CLPPs. A recently clear-felled site and an old clear-felled site were compared with a standing forest site. The activities of exoenzymes in the humus layer increased 12 months after clear-felling. During this period, the amount of fungi increased in the humus layer as compared with that in the standing forest soil. The utilization potential of carbohydrates increased more than the utilization potential of amino acids and carboxylic acids in the humus layer of the new felled site. Immediate effects in the humus layer were followed by a 1-year delay of minor effects in the mineral layer. We showed that instant structural polymer degradation is initiated by a burst of exoenzymes in the humus layer. This effect was still observed in the old felled site seven years after felling.

Introduction

Forest soil micro-organisms influence the nutrient cycling of the whole ecosystem. The decomposing community includes a wide range of individual species, which may respond very differently to changes in the environment. The death of sensitive microbes after disturbances in forest soil may favour the growth of better adapted microbes and cause changes in the microbial community and decomposition activity (Bååth *et al*. 1995, Hernesmaa *et al*. 2005, Heinonsalo and Sen 2007).

Under natural conditions carbon enters the soil as litter and dead roots, and through rhizodeposition; in the soil, these carbon compounds are mostly insoluble macromolecules. To be transportable into microbial cells, these polymers must be converted into smaller oligomers outside the cell membrane. Enzymes produced by micro-organisms are secreted outside their cytoplasmic membrane where these exoenzymes then act as catalysts (Priest 1984, Nannipieri *et al*. 1988). Few micro-organisms produce a complete set of exoenzymes capable of degrading native cellulose efficiently into smaller mono-

saccharides but micro-organisms that degrade cellulose usually also degrade hemicelluloses (Warren 1996). In addition to cellulose degrading cellulases, xylanase is the principal enzyme in primary litter degradation (Schinner 1995) and this enzyme degrades hemicelluloses by hydrolysing the xylan backbone (Béquin and Aubert 1994). Xylans are structural polymers and storage compounds in plants and are more easily degradable than cellulose.

Many exoenzymes are synthesised at very low rates during the exponential growth of microbes, and at a maximum rate during their stationary growth phase (Priest 1984). Exponential growth of bacteria in field conditions is uncommon and the production of enzymes that increase the availability of substrates is likely to be fully induced. Active enzymes may be maintained free in soil water or be attached to soil particles for long time periods after cell lysis (Nannipieri *et al*. 1988, Wittmann *et al*. 2000). Therefore, measured exoenzyme activities may not represent the current activity in the soil, but that of an earlier situation. Although the age of enzymes cannot be assessed under field conditions, an increase in enzyme levels that follows changed soil conditions is likely to indicate recent production.

In Finland, approximately 74% of regeneration felling is performed by clear-felling (Peltola 2001). Removal of trees decreases litter production and diminishes the carbon in the soil, while rain intensifies leaching of nutrients and humic compounds from the soil. The intensity of radiation reaching the soil surface increases the temperature in the topsoil, possibly drying it, while lower transpiration from the vegetation may result in an elevation of the groundwater level. Clear-felling of trees halts the carbon flow of photosynthetic products from canopy to roots and the associated microbial community. The microbial community then becomes dependent on the degradation of dead organic matter and carbon turnover by the developing herbaceous plant cover. Soil may also be compressed due to heavy logging machines. Forest treatment practices affect soil processes in many ways that can sometimes result in poor growth of tree seedlings during reforestation after clear-felling. Therefore it is important to know how felling affects chemical and biological processes in the soil.

The aim of this work was to study the impact of clear-felling on the number of bacteria and fungi in the forest soil and to investigate changes in soil microbial exoenzyme activities and the concurrent soil microbial carbon source utilization pattern and efficiency after clear-felling. We hypothesized that the composition and the carbon metabolism of the microbial community would change after clear-felling and that the organic matter degradation processes would be initiated by a burst of exoenzymes that attack structural polymers present in the soil. For two growing seasons we compared microbial activities in the coniferous forest humus and mineral soil layers of a standing forest, a new clear-felled site and a site that had been clear-felled six years before the onset of the study. We examined the extent and direction of the microbial activity shift that occurred in the soil after tree-felling.

Materials and methods

Study area, sampling and characterization of soil parameters

The field study area located in Nastola, in southern Finland (61°00´N, 25°50´E), was a boreal coniferous forest dominated by Scots pine (*Pinus sylvestris*). The total study area was approximately 1 hectare. The soil was a poorly developed podzol (Table 1). The deviations between replicates were high due to high organic matter content. In northern regions humic substances account for approximately 60% of soil organic matter (Stevenson and Cole 1999). These compounds are the results of biological and chemical transformations of plant, animal and microbial residues that are more stable than their precursors. Even in grasslands humic remains has been dated to be over 2000 years old by using ^{14}C (Anderson and Paul 1984). Podzolized soil with high amount of acidic humic substances is demanding matrix to study.

In October 1998 clear-felling was performed at the forest slope leading to lake Sammalisto (= felled site). The standing control site bordered the felled site on the slope. The site that had been clear-felled 6 years earlier (= old felled site) was bordering down the slope from the felled and

Table 1. Mean chemical, physical and biological variables (n = 3, ± SD) of boreal Scots pine forest humus and mineral soil layers in soil samples before and after the felling. Comparison before the felling was done between soils sampled in June 1998 (before felling) and from the 6 years earlier clear-felled site (old felled).

	Time after felling							
	Before		1 week (autumn)		9 months (summer)		12 months (autumn)	
	Old felled	felling	Standing	Felled	Standing	Felled	Standing	Felled
Humus								
pH (H_2O)	4.8 ± 0.0*	4.3 ± 0.1	4.8 ± 0.3	4.5 ± 0.1	4.0 ± 0.1	4.4 ± 0.1*	4.0 ± 0.1	4.7 ± 0.1*
Dry matter (%)	42.5 ± 9.0	36.9 ± 3.9	22.4 ± 1.9	29.2 ± 2.2	58.5 ± 6.2	66.9 ± 5.2	42.9 ± 5.8	43.4 ± 1.1
Organic matter (%)	56.8 ± 21.5	74.0 ± 6.4	91.2 ± 1.6	76.5 ± 7.5	58.5 ± 12.0	30.6 ± 2.7	55.8 ± 18.3	60.6 ± 4.7
Total C (mg g^{-1} dw)	361 ± 5.0	371 ± 45	483 ± 5.2	206 ± 89.0 *	284 ± 74.0	135 ± 11.0	316 ± 113	318 ± 43.6
Total N (mg g^{-1} dw)	13 ± 0.0	12 ± 2.2	13.0 ± 0.6	7.0 ± 3.1	9.6 ± 2.4	5.5 ± 0.7	9.5 ± 2.7	11.8 ± 1.5
PO_4-P (µg g^{-1} dw)	2.8 ± 0.2	2.3 ± 1.4	25.9 ± 10.7	20.5 ± 15.2	4.4 ± 3.6	5.0 ± 2.3	1.2 ± 0.0	26.5 ± 6.6*
NH_4-N (µg g^{-1} dw)	2.0 ± 0.1	7.3 ± 2.5	1.7 ± 0.1	1.7 ± 0.1	14.0 ± 6.9	35.8 ± 18.3	2.8 ± 1.5	109.3 ± 50.1
NO_3-N (µg g^{-1} dw)	0.1 ± 0.0	0.1 ± 0.0	< 0.1	< 0.1	< 0.1	< 0.1	0.4 ± 0.1	0.4 ± 0.0
Total fungi $\log_{10}$ (cfu g^{-1} dw)	nd	nd	nd	nd	4.2 ± 0.16	4.3 ± 0.16	5.5 ± 0.18	5.2 ± 0.26
Total heterotrophs $\log_{10}$ (cfu g^{-1} dw)	6.2 ± 0.2	6.0 ± 0.3	6.8 ± 0.17	6.9 ± 0.25	5.9 ± 0.13	6.6 ± 0.45	5.9 ± 0.23	6.5 ± 0.13
Mineral								
pH (H_2O)	5.0 ± 0.1	4.8 ± 0.2	4.6 ± 0.0	5.3 ± 0.1	4.7 ± 0.1	4.5 ± 0.1	4.7 ± 0.0	4.8 ± 0.2
Dry matter (%)	60.7 ± 2.9*	77.6 ± 2.5	72.7 ± 1.4	77.5 ± 4.6	81.0 ± 2.4	81.2 ± 3.3	76.2 ± 0.7	74.3 ± 3.0
Organic matter (%)	14.7 ± 1.7*	8.1 ± 1.4	8.9 ± 0.8	6.5 ± 1.0	12.1 ± 2.3	8.8 ± 1.3	9.8 ± 0.2	7.7 ± 1.5
Total C (mg g^{-1} dw)	101 ± 17*	35 ± 7	24.0 ± 3.1	46.3 ± 6.5*	56.6 ± 10.7	42.1 ± 7.3	44.5 ± 3.1	39.1 ± 7.3
Total N (mg g^{-1} dw)	3.3 ± 0.9	1.2 ± 0.3	0.8 ± 0.2	1.3 ± 0.2	2.0 ± 0.4	1.6 ± 0.2	1.7 ± 0.1	1.5 ± 0.3
PO_4-P (µg g^{-1} dw)	4.9 ± 1.1	2.6 ± 1.9	0.4 ± 0.3	2.6 ± 0.6*	0.3 ± 0.1	0.3 ± 0.2	0.1 ± 0.0	0.2 ± 0.0
NH_4-N (µg g^{-1} dw)	1.7 ± 0.5	1.2 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	10.1 ± 9.4	4.1 ± 1.3	1.0 ± 0.1	5.0 ± 0.6*
NO_3-N (µg g^{-1} dw)	0.1 ± 0.0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.8 ± 0.0	0.7 ± 0.0
Total fungi $\log_{10}$ (cfu g^{-1} dw)	nd	nd	nd	nd	3.6 ± 0.3	3.8 ± 0.1	3.1 ± 1.5	2.8 ± 1.4
Total heterotrophs $\log_{10}$ (cfu g^{-1} dw)	5.3 ± 0.1	5.0 ± 0.2	6.1 ± 0.2	6.9 ± 0.25	5.7 ± 0.1	5.7 ± 0.4	5.6 ± 0.1	5.4 ± 0.3

* Each time point was treated statistically as a separate observation and statistically significant differences (t-test p<0.05) during the time of sampling between standing before felling and old felled or standing and felled are indicated.
nd = not determined.

standing forest control sites, and was closer to the lake. All adjacent sites originally represented the same forest type and were not further treated during the period of investigation.

Soil samples of humus and mineral layers from the standing, new felled and old-felled sites were collected with a Westman core (Westman 1995). The humus layer was separated from the mineral layer by the colour of the layer. Three replicate 200 g composite samples were collected from each marked study site (1.5 m × 1.5 m) by combining randomly taken cores after separation of the humus and mineral layers. The samples were sieved (mesh diameter 1.5 cm for humus and 0.7 cm for mineral soil) and analyses were initiated within 24 h after sampling. Control measurements and comparison before the felling of the new-felled site was done in summer 1998. All three sites were sampled again in autumn 1998, one week after clear-felling, and again the next summer and autumn in 1999. In 2000 only the humus layer was sampled (20 and 24 months).

Dry matter contents were determined at 105 °C for 16 h and organic matter contents were determined as ignition loss (weight %) after 4 h at 550 °C. pH was determined by stirring 4 g of moist soil with water (1:2) for 1 h and the total nutrients were measured by an automatic analyzer (CHN-600, LECO). Ten g moist soil were stirred in water (1:5) for 2 h for PO_4-P analyses and stirred in 2 M KCl (1:5) for 2 h for NH_4-N and NO_3-N and analysed with Lachat (Quikchem 8000).

Exoenzyme assays

Exoenzyme activities were measured according to Wittmann *et al*. (2000) using 4-methylumbelliferyl (MUF) fluorogenic model substrates (Fluoroskan Ascent, Labsystems, Finland). Substrates for phosphomonoesterase, butyrate-esterase, β-glucosidase, β-cellobiosidase, N-acetyl-glucosamidase and xylosidase (Sigma, St. Louis, MO, USA) were used at final concentrations of 1 mM as indicators for phosphatase, esterase, two cellulases, chitinase and hemicellulase activity, respectively. The fluorescence level was measured at 30 °C on-line in 96-well microtitre plates as described by Hernesmaa *et al*. (2005). Measurements were taken within 24 h of sampling at soil *in situ* pH. Each well contained 50 μl of sieved soil (no soil suspension was made), sterile water and substrate stock solution. Enzyme activities were measured online within 1 h after substrate addition. The activities during the linear phase of the reaction are reported as the rate of MUF production. After the reaction, 20 μl 1 M tris-HCl (pH 8.9) was added to increase pH to produce optimal fluorescence, which was then measured and calculated as described by Wittmann *et al*. (2000).

Community level physiological profiles

Community level physiological profiles (CLPPs) in humus and mineral soil layers were determined with Biolog GN plates (Biolog, Inc., Hayward, USA). The microbes were extracted as described below, then further diluted to 10^{-2} and inoculated onto micro plates containing 95 different carbon sources and a water control. Plates were incubated for 4 d at 20 °C and absorbencies indicating utilization of each carbon source were measured daily using a Biolog Workstation as described by Björklöf *et al*. (2003). For CLPPs, the areas of the curves representing absorbency *versus* time of each substrate were used for analysis. The efficiency of utilization of carbon compounds by the different microbial communities in the samples was determined by comparing the mean areas of the substrates. The number of bacteria in the inoculums influences the utilization efficiencies. Therefore 4´,6-diamidino-2-phenylindole (DAPI) counts (Tuomi *et al*. 2004) were used to determine the bacterial numbers in all samples. The variation between the numbers of inoculated cells was less than eight times in the humus layers (total cell counts between 3.2×10^8 cells g^{-1} dw to 2.5×10^9 cells g^{-1} dw) and less than six times in the mineral soil layers (1.3×10^8 cells g^{-1} dw to 7.9×10^8 cells g^{-1} dw).

Enumeration of micro-organisms

Bacteria were extracted as described by Laine *et al*. (1997) by shaking (20 min, 200 rev. min^{-1}) 4 g of soil (w/w) in 36 ml buffer (0.9% NaCl, 1 ml

of 10.4% $Na_5P_3O_{10}$ and 100 μl 2% Tween 80) at room temperature before serial dilution in water and plating. To enumerate the total plate count of aerobic heterotrophic bacteria, 1/5 tryptone-glucose-yeast agar plates (Laine *et al*. 1997) were incubated for 6 d. Pectin and cellulose decomposers were incubated for 7 d on media described by Chatterjee and Starr (1972) and Andro *et al*. (1984) (in both media the second carbon source was diluted 100×), chitin decomposers on chitin media for 12 d (Sarby 1992) and lignin decomposers on lignin media for 21 d (Hayakawa *et al*. 1996). All plates contained 100 μg cycloheximide ml^{-1} to prevent fungal growth. For the extraction of fungi separate dilution series (shaken for 2 h) were made. Total fungi were incubated in 1/5 potato-dextrose-agar plates for 7 d. Pectin, cellulose, chitin and lignin plates were used as above except that the plates contained 60 μg ml^{-1} of ampicillin and 60 μg ml^{-1} of streptomycin to prevent bacterial growth, and no cycloheximide was added. All plates were incubated in the dark at 20 °C.

Statistical analysis

Differences between the standing forest soil and clear-felled forest soils were compared with one-way ANOVA (SPSS, Chicago, Ill.). The data set was not treated statistically as repeated measurements over time because the samplings were performed in different seasons and different persons performed the sampling at different times. These factors and the fact that humus soil is a very complex and non-homogenous substrate, resulted in considerable variation between the time points. Instead the standing *vs*. the felled or the felled (before felling) and old felled were tested pair wise for each time point separately. Principal component analyses were performed using Matlab® software, using correlation matrices.

Results

Chemical parameters of the soil

As expected, the humus and mineral layers had very different soil structures and properties in the standing forest soil (Table 1). The mean organic matter content of the mineral layer was 10%, whereas the organic matter content of the humus layer was 70%. The high organic matter content of the humus layer kept it moister than the mineral soil. The pH was normally higher in the mineral layer than in the humus layer. The pH was significantly higher at the old-felled site than at the control site before felling and was higher 12 months after felling as compared with that at the standing site (Table 1). The concentrations of soluble NH_4^+-N increased in the humus and mineral layer 12 months after felling. The concentrations of soluble PO_4^{3-}-P in the humus layer were elevated 12 months after felling (Table 1). At the old-felled site concentrations of soluble nutrients were more related to the control standing site than the new-felled site (data not shown).

Exoenzymes

Tree-felling activated exoenzyme activities in the soil humus layer. All measured exoenzyme activities were elevated already one week after felling as compared with those at the standing site. Significantly higher β-glucosidase and cellobiosidase activities 12 months after felling indicated the presence of higher cellulose decomposition activity at the felled site as compared with that at the standing site (Fig. 1). These cellulase related activities were still elevated 20 and 24 months after felling, even though their level was about half that of the activities in 1999 after 12 months (data not shown). In the new felled area the chitinase activity was significantly elevated nine months after felling. At the same sampling time the corresponding activity at the standing site soil also increased and was elevated in 2000 when activity at the felled site had already decreased (data not shown).

The activities of specific enzymes in the mineral layers at the different sites were quite similar to each other (Fig. 2). The activities at the standing site tended to be the same as, or higher than activities at the clear-felled sites. In the mineral layer at the old-felled site the chitinase activity appeared to be reduced as compared with that at the other sites.

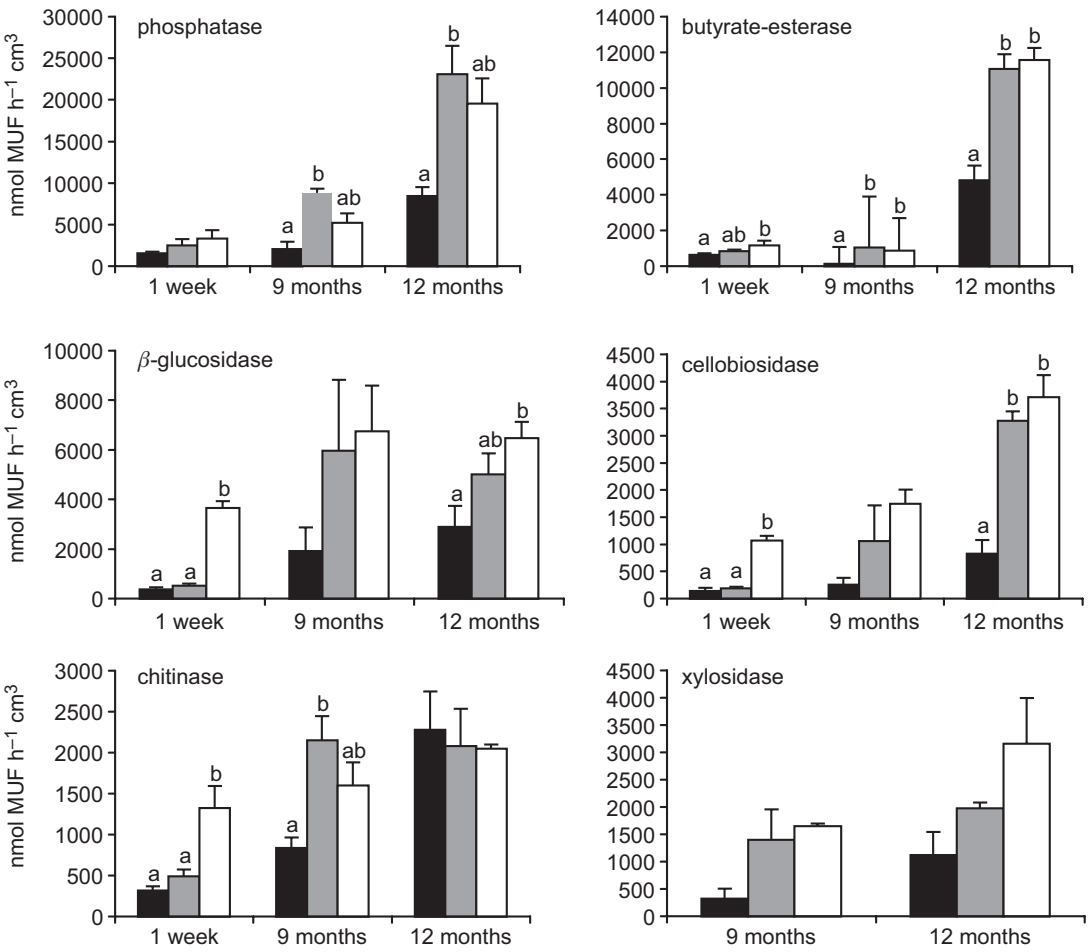

Fig. 1. Mean exoenzyme activities (n = 3, error bars = SE) measured in soil at native pH using 4-methylumbelliferyl (MUF)-substrates in the humus layer of the soil samples from boreal Scots pine forest soil of the standing (black bars) felled (grey bars) and old-felled (white bars) soil sites. Statistically significant differences between the sites (p < 0.05) are indicated with letters.

At the old-felled site, the β-glucosidase, cellobiosidase and chitinase activities of the humus layers were significantly elevated as compared with those at the standing site (Fig. 1).

Community level physiological profiles

The microbial community's potential to utilize different types of carbon substrates in the humus layer was enhanced one week after felling as compared with that at the standing site (Table 2). Nine months after felling, all groups of carbon substrates were utilized more efficiently in the humus layer of the new-felled site as compared with the standing sites (Table 2). In the mineral layer, similar enhanced activities were observed at the felled site 12 months after clear-felling. The utilization efficiencies of specific carbon substrates that are linked to decomposition of polysaccharides were compared between standing- and felled-site soils (Table 3). In the humus layer, the utilization efficiency of many of these compounds was already higher at the felled site as compared with that at the standing site already one week after felling. This observation was especially clear for the utilization of cellobiose (dimer of cellulose) and N-acetyl glucosamine

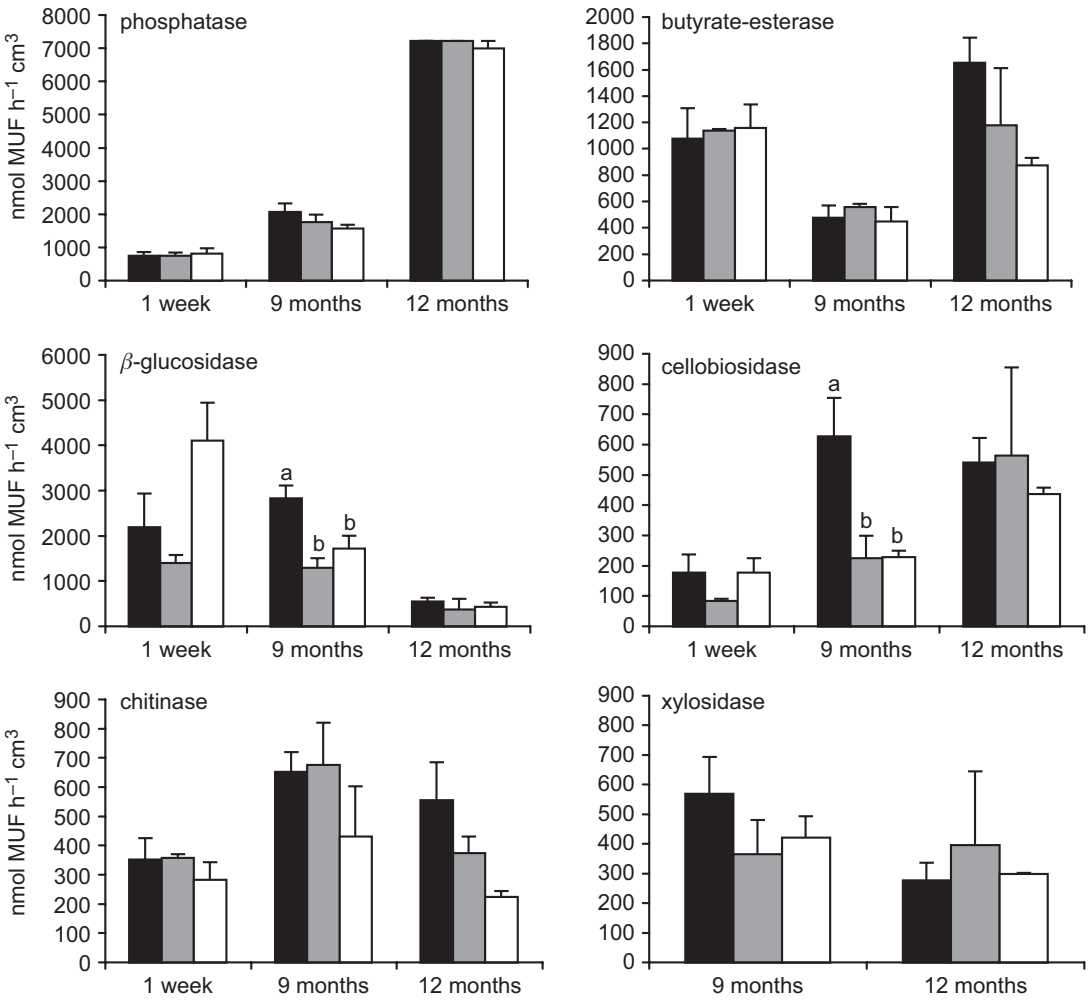

Fig. 2. Mean exoenzyme activities (n = 3, error bars = SE) measured in soil at native pH using MUF-substrates in the mineral layer of the soil samples from boreal Scots pine forest soil of the standing (black bars), felled (grey bars) and old-felled (white bars) soil sites. Statistically significant differences between the sites ($p < 0.05$) are indicated with letters.

(the chitin monomer) for which carbon utilization potentials in the humus layer were already two times higher at the felled site as compared with those at the standing site already one week after felling (Table 3, ratio = 0.5). This observation was reflected in the mineral soil layer from nine months onwards after felling, where cellobiose utilization was higher at the felled site and N-acetyl glucosamine utilization was more than 100 times higher at the felled site 12 months after felling. Multivariate statistics (principal component analysis) did not reveal further differences between the sites (data not shown).

Enumeration of micro-organisms

Variations between replicate soil samples were high especially among the bacterial degrader populations (Fig. 3) and fungal degrader populations (Fig. 4) as measured by plate counts. Statistically significant differences between soil samples from the felled area and the standing control were found mostly between bacterial degraders in the humus (Fig. 3A) and mineral (Fig. 3B) soil layers one week or nine months after felling. The numbers of bacterial chitin decomposers in the humus layers of the new felled sites were

Table 2. Efficiency of substrates utilization* at community level physiological profiles by microbial communities extracted from humus and mineral soil layers after felling. Substrates are grouped according to Campbell *et al.* (1997). Statistically significant differences between the sites at a specific time point ($p < 0.05$ or less) are indicated with letters.

		Humus						Mineral					
Time after felling	Site	Carbohydrates	Carboxylic acids	Aminoacids	Amides	Polymers	Other	Carbohydrates	Carboxylic acids	Aminoacids	Amides	Polymers	Other
1 week	Standing	43 ± 6	21 ± 3	27 ± 4	19 ± 5	11 ± 7	24 ± 2^{a}	36 ±14	20 ± 7	23 ± 9	10 ± 6	8 ± 8	27 ± 6
(autumn)	Felled	56 ± 1	27 ± 1	34 ± 1	26 ± 3	17 ± 2	38 ± 4^{b}	32 ± 7	20 ± 2	25 ± 2	15 ± 5	11 ± 6	22 ± 3
	Old felled	50 ± 7	27 ± 5	36 ± 7	26 ± 2	20 ± 6	30 ± 6^{ab}	36 ± 11	25 ± 4	31 ± 6	21 ± 7	14 ± 9	20 ± 11
9 months	Standing	45 ± 5	19 ± 5^{a}	21 ± 9	13 ± 6	5 ± 1^{ab}	27 ± 5	25 ± 7	26 ± 9	28 ± 9	18 ± 13	3 ± 3	9 ± 6
(summer)	Felled	48 ± 15	30 ± 2^{b}	36 ± 3	27 ± 7	10 ± 4^{b}	34 ± 15	26 ± 18	23 ± 5	25 ± 7	20 ± 5	1 ± 2	13 ± 22
	Old felled	48 ± 6	26 ± 4^{ab}	32 ± 6	18 ± 6	4 ± 2^{a}	32 ± 12	34 ± 10	23 ± 3	26 ± 7	19 ± 8	2 ± 2	12 ± 10
12 months	Standing	48 ± 28	22 ± 12	26 ± 19	16 ± 14	7 ± 4	35 ± 8	15 ± 2^{a}	18 ± 2^{a}	21 ± 3^{a}	6 ± 2	4 ± 1	3 ± 3^{a}
(autumn)	Felled	77 ± 9	37 ± 2	48 ± 5	37 ± 5	16 ± 2	45 ± 5	65 ± 10^{b}	32 ± 3^{b}	36 ± 6^{b}	25 ± 3	5 ± 1	39 ± 4^{b}
	Old felled	67 ± 7	33 ± 2	41 ± 5	30 ± 7	22 ± 24	45 ± 8	55 ± 10^{b}	33 ± 6^{b}	41 ± 8^{b}	24 ± 13	6 ± 2	33 ± 23^{b}

* The utilization efficiency is expressed as the mean areas ($n = 3$) ± SD of the absorbance curves over the incubation time.

higher than at the standing control nine months after the felling. In the mineral layer at the old-felled site, the numbers of cellulose, chitin, and pectin decomposing bacteria and fungi were often higher than at the felled or standing site (Fig. 3B and 4B).

The amounts of some fungal degraders in the humus layer at the newly felled site were significantly higher ($p > 0.005$) 20 months after felling than at the standing control site. The mean number ($n = 3$) of fungal chitin degraders was 4×10^4 CFU g^{-1} organic matter at the newly felled site as compared with 5×10^3 CFU chitin degraders g^{-1} organic matter at the standing site. The corresponding mean numbers ($n = 3$) of fungal lignin degraders were 2×10^5 CFU g^{-1} organic matter at the newly felled site as compared with 4×10^4 CFU lignin degraders g^{-1} organic matter at the standing site. The mean number of fungal cellulose degraders was 2×10^5 CFU g^{-1} organic matter at the newly felled site as compared with 1×10^3 CFU cellulose degraders g^{-1} organic matter at the standing site.

Discussion

We studied the activity responses in the humus and mineral layers after clear-felling and compared them with those in the standing control forest soil. An old-felled site in the same area was monitored to study the prolonged effects of clear-felling. Tree-felling rapidly increased the utilization potential of several carbon sources and activated microbial polymer degradation in the soil humus layer. In particular, butyrate-esterase, β-glucosidase, cellobiosidase and chitinase showed significantly elevated activities during first growing season after felling as compared with those at the standing site. The cellulose decomposition activity and carbon substrate utilization potential were elevated in the humus layer at the old-felled site seven years after felling and the activity profile at the old-felled site resembled more that at the new felled site than that at the standing site. In the mineral layer, bacterial and fungal chitin, pectin and cellulose decomposers and the carbon substrate utilization

Table 3. Comparison of microbial community efficiency to degrade selected C substrates reflecting wood decomposition in humus and mineral layers after felling. Comparisons are based on the ratio between utilization efficiencies at the standing site and the felled site at the corresponding sampling times.

			Standing/felled ratio of utilization efficiency* (time after felling)		
Soil layer	Component of	Carbon substrate	1 week (autumn)	9 months (summer)	12 months (autumn)
Humus	cellulose, hemicellulose, xylose	glucose	0.7	0.9	0.6
	cellulose	cellobiose	0.5	0.5	0.4
	xylose, hemicellulose	galactose	0.8	1.0	0.7
	pectin	galacturonic acid	0.7	0.8	0.7
	pectin	rhamnose	0.8	0.7	0.8
	xylose	L-arabinose	0.6	0.7	0.6
	xylose	D-mannose	0.7	1.0	0.7
	chitin	N-acetyl-glucosamide	0.5	0.8	0.7
Mineral	cellulose, hemicellulose, xylose	glucose	1.0	1.2	0.6
	cellulose	cellobiose	1.3	0.3	0.2
	xylose, hemicellulose	galactose	1.1	1.7	0.2
	pectin	galacturonic acid	0.8	1.1	0.3
	pectin	rhamnose	0.8	0.7	0.1
	xylose	L-arabinose	0.6	0.7	0.2
	xylose	D-mannose	1.3	0.9	0.3
	chitin	N-acetyl-glucosamide	0.1	0.2	0.0

* Mean ratio ($n = 3$) in standing to felled soil. The utilization efficiency is expressed as the area of each absorbance curve over incubation time.

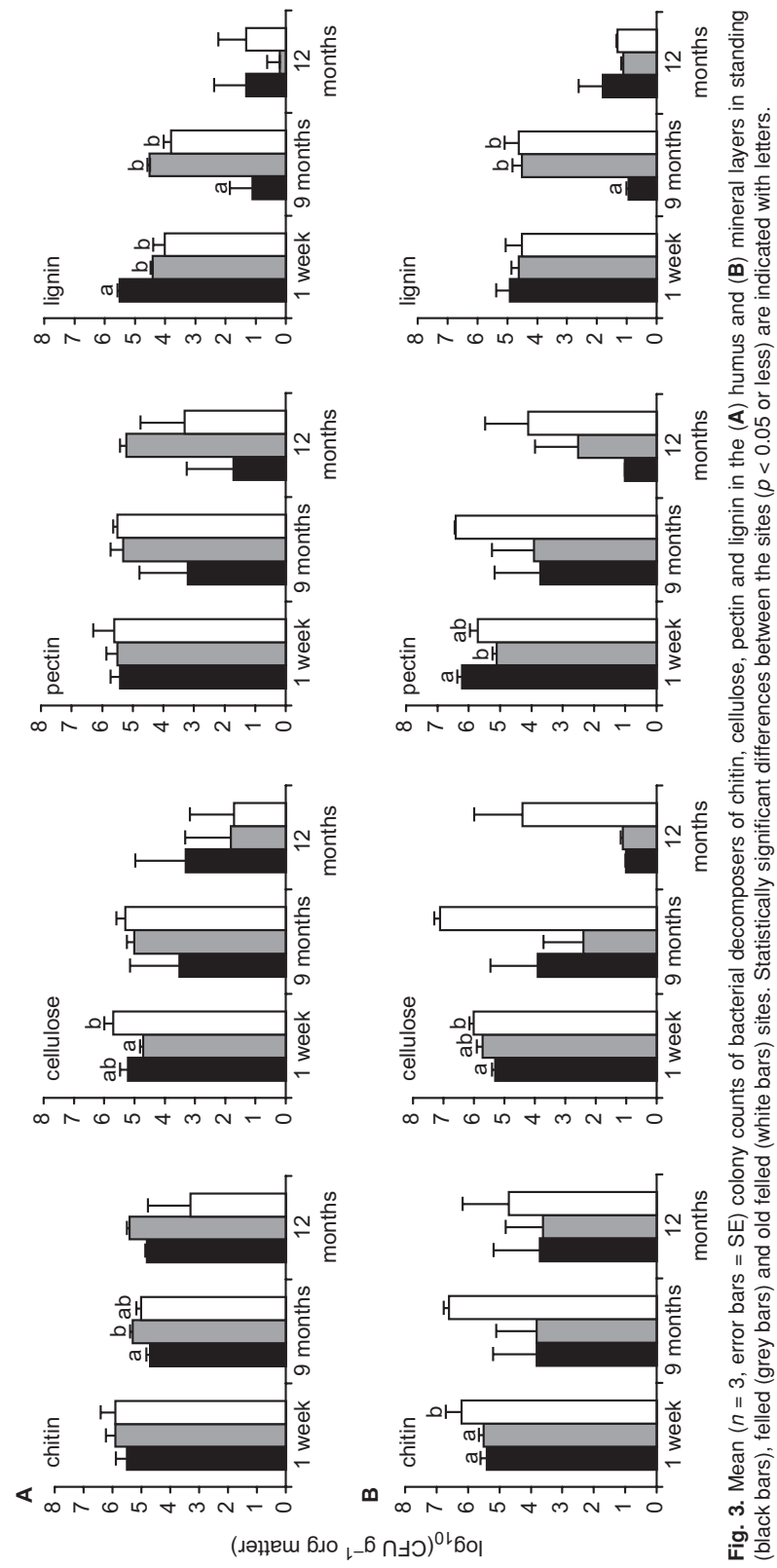

Fig. 3. Mean (n = 3, error bars = SE) colony counts of bacterial decomposers of chitin, cellulose, pectin and lignin in the (**A**) humus and (**B**) mineral layers in standing (black bars), felled (grey bars) and old felled (white bars) sites. Statistically significant differences between the sites ($p < 0.05$ or less) are indicated with letters.

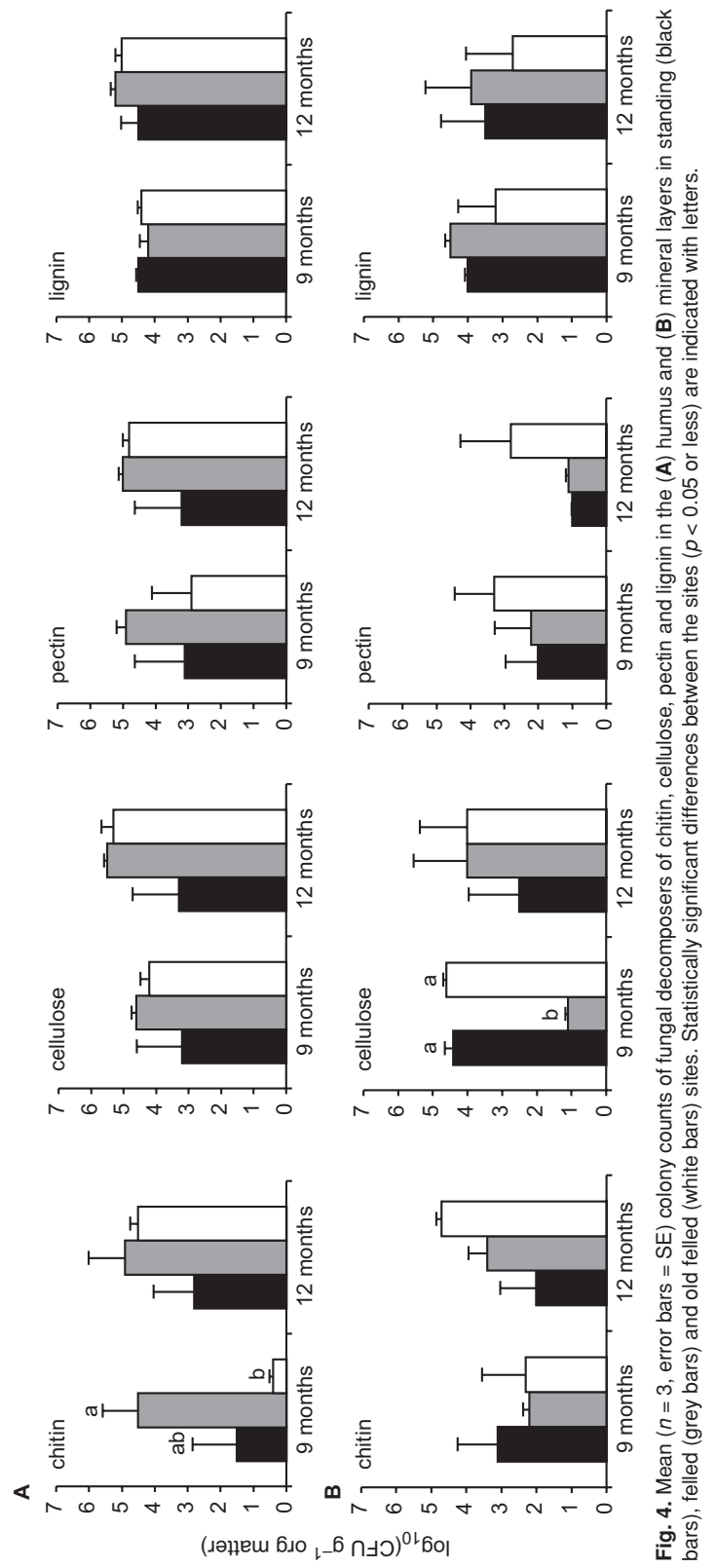

Fig. 4. Mean (n = 3, error bars = SE) colony counts of fungal decomposers of chitin, cellulose, pectin and lignin in the (**A**) humus and (**B**) mineral layers in standing (black bars), felled (grey bars) and old felled (white bars) sites. Statistically significant differences between the sites ($p < 0.05$ or less) are indicated with letters.

potential were often higher at the old-felled site as compared with those at the other sites but on the other hand chitin exoenzyme activity was often lowest.

The maxima of the measured exoenzyme activities at the felled site were observed 9 to 12 months after felling. These activities were elevated already one week after felling but the differences were not statistically significant. The exoenzyme activity maxima in the humus layer (Fig. 1) coincided with an increase in the colony numbers of fungal decomposers (Fig. 4a). Fungi are considered to be the primary decomposers in podzol soil and a correlation between fungal biomass and MUF-chitinase activity has been demonstrated (Miller *et al*. 1998). We observed enhanced chitin monomer utilization potential after felling, especially in the mineral layer. This may indicate consumption of chitin, a major component of the fungal cell wall, and thus probably the death of mycorrhiza. The amounts of active ectomycorrhizal roots and fungal biomass have been observed to decrease after felling (Bååth 1980, Harvay *et al*. 1980). However, ectomycorrhizas can remain viable in the decaying roots several years after clear-felling (Hagerman *et al*. 1999). The culturable number of fungal cellulose decomposers was also higher in the humus layer of the old-felled site as compared with that of the standing site. In the mineral layer, bacterial chitin, cellulose and pectin decomposer numbers were insignificantly higher at the old-felled site as compared with those at the standing site.

Twelve months after clear-felling, the exoenzyme activities of β-glucosidase and cellobiosidase representing cellulose degradation were significantly enhanced in the humus layer, indicating that cellulose is degraded initially and more lignified components are degraded later. There is not much information on the instant effect of clear-felling on the microbial community activity but long term studies have shown that harvest treatments may reduce enzyme activities from 10% to 30% in litter (Waldrop *et al*. 2003, Hassett and Zak 2005) or have no significant effect (Maassen *et al*. 2006). In our study the cellulose decomposition activity and number of decomposers were still elevated in the humus layer at the old-felled site as well as the substrate utilization potential in both layers.

Readily degradable compounds such as amino acids and monosaccharides have been postulated to be a part of the fast pool of carbon, which is rapidly (8–10 times per year) cycled through microbial cells, grazers and plants. It has been estimated that more recalcitrant organic matter compounds, including humic substances, are cycled through microbes once every 10–100 years (Coleman *et al*. 1983). We observed that the differences in the substrate utilization patterns in the humus layer one week after felling were mainly caused by enhanced usage of carbohydrates, but later mostly amino acids and carboxylic acids were consumed. Dai *et al*. (2001) showed that carbohydrates comprise 32%–49% of the total C in topsoil and the amount of carbohydrates decreases with soil depth. After the removal of trees, the dissolved organic matter fractions are likely to be transported with water to the deeper soil layers. We observed that the increase in the potential for using carbohydrates in the mineral layer was delayed as compared with that in the humus layer.

According to our results the carbon substrate utilization potential in the mineral layer was enhanced one year after felling but the exoenzyme activities in the mineral soils were not. Exoenzyme activities were not significantly enhanced in the mineral layer during the study. Exoenzyme activities were always measured within 24 h of sampling at the native pH with no disturbance other than the addition of water-suspended MUF-substrate to the soil sample. As active exoenzymes may be maintained in the soil, the measured activity may also represent activity produced earlier and not reflect only the conditions and presence of microbes in the sampling areas at the time of sampling. On the other hand, due to the incubation step in CLPP the reactions represent events taking place after sampling and reflect only the potential of the microbial community, not their real activity at the time of sampling. The incubation step needed for analysis may alter the active population of the samples (Smalla *et al*. 1998) and the role of fast growing bacteria may be overrepresented (Winding and Hendriksen 1997). Therefore, results generated by these two methods, CLPP and fluorogenic MUF-substrate utilisation analysis, complement each other.

In the present study the carbon substrate utilization potential of the soil microbial community in the humus layer was enhanced only one week after the clear-felling of the trees. E. Heikkinen (pers. comm.) showed that during a 125-day laboratory incubation the release of N from fresh *Pinus* debris was the highest on day one. Growth in the boreal forest is generally limited by the availability of N from slow mineralization (Näsholm *et al*. 1998, Stevenson and Cole 1999). Release of N after felling may activate the consumption of other available substrates. The observed increase in the potential to use readily degradable compounds one week after clear-felling is therefore not surprising. The soil microbial activity in the humus layer was enhanced and stayed elevated probably because of the new carbon source after the growth of ground vegetation, and the formation of new root systems and litter.

Forest harvesting substantially alters the physical environment in soil, as well as the amount and biochemical characteristics of plant detritus, and these changes are likely to alter the microbial community composition and function. In this study, the pH of the soil was 0.4–0.7 units higher at the new felled site than at the standing site during the first growing season after the clear-felling. Losses of soil nitrogen (Holmes and Zak 1999) as well as increases in pH after felling have previously been reported (Pietikäinen and Fritze 1995, Siira-Pietikäinen *et al*. 2001). An increase in soil pH after clear-felling is probably due to the release of cations from the decomposable organic matter (Berdén *et al*. 1987) and changes in detritus formation; the cease of pines debris and enhanced growth of grass. We also observed that *Vaccinium vitis-idaea* was still the most common species three years after the felling, but *Epilobium angustifolium* appeared as a pioneer plant in the newly felled area. *Convallaria majalis* disappeared totally and the previously common species *Maianthemum bifolium* and *Trientalis europae* were heavily reduced (J. Oksanen pers. comm.).

The microbial activity in the humus layer reacted more readily to tree-felling than the mineral layer and the maintenance of the humus layer is thus extremely important for the recovery of the forest ecosystem. We were not able to detect significant increases in nitrogen levels in the humus layer due to felling but herbaceous plant cover developed gradually at the felled site, probably benefiting from the released nitrogen. The new vegetation cover could provide the microbial community with new carbon sources from the newly developed root system and litter.

The changes in the microbial activity show that the microbial community is able to adapt fast to dramatic events in the ecosystem such as clear-felling. This ability is also needed when replanting forest trees in clear-cut areas. The re-establishment of tree-ectomycorrhizal interactions is of major importance for the success of newly planted tree seedlings when they compete with the herbaceous vegetation cover for nutrients and light.

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