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Faculty of Agriculture and Forestry
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Front cover: The forest stand in Patvinsuo National Park in July one month after the fire. Photograph by J. Rahkonen.

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Contents

Original publications

This thesis is based on the following articles, referred to in the text by their Roman numerals:

I Pietikäinen, J. and Fritze, H. 1993. Microbial biomass and activity in the humus layer following burning: short-term effects of two different fires. Canadian Journal of Forest Research 23: 1275-1285.

II Fritze, H., Pennanen, T. and Pietikäinen, J. 1993. Recovery of soil microbial biomass and activity from prescribed burning. Canadian Journal of Forest Research 23: 1286-1290.

III Pietikäinen, J. and Fritze, H. 1995. Clear-cutting and prescribed burning in coniferous forest: comparison of effects on soil fungal and total microbial biomass, respiration activity and nitrification. Soil Biology and Biochemistry 27: 101-109.

IV Pietikäinen, J., Hiukka, R. and Fritze, H. 1999. Does short-term heating of forest humus change its properties as a substrate for microbes? Soil Biology and Biochemistry (in press).

V Pietikäinen, J., Kiikkilä O. and Fritze, H. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Submitted manuscript.

Studies I, III, IV and V were carried out mainly by J. Pietikäinen. R. Hiukka was responsible for the spectroscopic analyses in Study IV. The work in Study II was done in co-operation with T. Pennanen.

Definition of some fire-related terms used in this study:

Ash. The mineral-rich powdery residue remaining after a fire (Jones et al. 1997).

Charcoal. Any black-coloured plant-derived material that has had its chemical composition and ultrastructure significantly altered as a result of heating in a fire, and retains recognisable anatomic structure of the parent plant, even if only in a fragmentary form (Jones et al. 1997).

Prescribed burning. The controlled use of fire to achieve specific forest management objectives, e.g. reduction of fire hazard, control of competing vegetation, creation of seedbeds and planting spots, and overall improvement of the efficiency of silvicultural operations by removing impediments to reforestation and stand management. Two types of prescribed burning are used in forestry: underburning, i.e. burning under mature forest canopies, and slash burning, i.e. a method of disposing of logging residue (Walstad et al. 1990). In this study the term 'prescribed burning' always refers to slash burning.

Wildfire. A sweeping and destructive conflagration (Webster's Third.. 1986). A fire that burns rapidly and is hard to extinguish (The Random House Dictionary 1978). Wildfire also includes forest fires.

Ground fire. Fire which consumes the organic matter beneath the surface litter of the forest floor, spreading within rather than on top of the organic mantle (Brown and Davis 1973).

Surface fire. Fire which burns the surface litter and debris of the forest floor as well as the low vegetation; fire behaviour is variable depending on conditions, may sometimes reach into the tree crowns (Brown and Davis 1973).

Crown fire. Fire which travels through the top layers of trees or shrubs, more or less independent of surface fire (Brown and Davis 1973).

1. Introduction

1.1 Occurrence of fires

Already in the Carboniferous period there were adequate numbers of terrestrial plants, photosynthesis-derived oxygen in the atmosphere and lightning to provide ignition of the biomass; and accordingly, charcoal layers as indicators of ancient fires have been recorded in fossils dating back to the boundary between Devonian and Carboniferous (Komarek 1973, Jones and Rowe 1999). Thus, as long as there has been terrestrial vegetation, there have been wildfires. This can also be seen as an evolutionary relationship; plants and other species inhabiting terrestrial environments have in the course of time at least co-existed, if not co-evolved with more or less frequent fires (Pyne 1996).

At present, the natural dynamics of wildfires is strongly controlled by only one species, *Homo sapiens*. The invention of deliberate fire ignition and its control by man started the anthropogenic modification of the biosphere (Pyne and Goldammer 1997). Since then, fire has been used for hunting, cooking, landscape management, farming, pastorization, forestry and reduction of fire hazard. The control and use of fire for human welfare has strongly interfered with the natural fire cycle. In many regions of the world both wildfires and man-made (i.e. prescribed) fires are at the present more frequent than they would be naturally. The boreal forests of North America and Siberia, with a continental climate, are characterised by large crown fires, which are caused either by humans or by lightning. In maritime regions, fires are less frequent and of lower intensity (Johnson 1992). The modern, strict fire suppression practised in e.g. Fennoscandia has been able to reduce the annually burned forest area and lengthen the fire interval (Zackrisson 1977).

Boreal coniferous forests are, by nature, fire-prone because of the structure of the forest canopy and the type of litter the trees produce. In general, conifers burn more easily than deciduous trees (Saari 1923) because the needles of conifers are drier than leaves, and they contain larger amounts of volatile organic compounds. In addition, the litter layer is rich in relatively fine fuels, which are intermingled with

a loosely packed moss layer; this promotes ignition (Schimmel and Granström 1997). The seasonal weather pattern includes a dry summer when lightning appears (Chandler et al. 1983). In Finland and Sweden the largest number of lightning ignitions occur in July (Saari 1923, Granström 1993). A wildfire is usually lit in the humus layer at the base of a lightning-struck tree (Granström 1993) and consequently the moisture content of the humus layer determines the number of lightning-ignited fires (Flannigan and Wotton 1991, Frandsen 1997).

Since factors determining the intensity and frequency of burning vary between sites, it is not relevant to try to determine one average value for the fire cycle in the boreal coniferous forest. Fire-return intervals ranging from 30 to 250 years have been reported for boreal coniferous forests, and the fire frequency has been shown to depend on climate, quality of fuel and vegetation, tree species, moisture status, mean waterbreak distance, topography and degree of human impact (Zackrisson 1977, Engelmark 1984, Masters 1990, Larsen 1997, Lehtonen and Huttunen 1997, Larsen and MacDonald 1998). Also the successional stage of the forest stand determines the risk of burning. Fires seldom spread in forest stands younger than 25 years and recently burned areas can actually act as fire breaks (Schimmel and Granström 1997). Some forests, e.g. in damp depressions or surrounded by bogs, have never experienced fire.

In modern forestry, wildfires are treated as an enemy, because fire consumes and destroys valuable timber. This has led to intensive fire suppression, the effectiveness of which is demonstrated by the small area burned annually. In Finland the number of forest fires in 1997 was 1409, but they were very restricted in size, as they burned only a total area of 841 ha (Finnish Statistical Yearbook of Forestry 1998, p. 90). However, as a forestry practice, fire is used for preparing clear-cut sites for forest regeneration, i.e. prescribed burning. In Finland, the use of prescribed burning first became very popular during the 1930's and later in the 1950's and 1960's, when up to 30 000 ha were burned annually (Viro 1969). Since then the prescribed burned area has declined to 1000-2000 ha per year (Finnish Statistical Yearbook of Forestry 1998, p. 115).

Although prescribed burning resembles wildfire in many respects, some noteworthy differences do exist. The main difference is in the

pre-fire vegetation of the site and the amount and quality of fuel consumed by the fire. Wildfires cover a wider range of intensities, from low-intensity ground fire to stand-replacing crown fire, while prescribed burning is conducted as a low intensity fire. A moderate wildfire usually kills deciduous trees and Norway spruce (*Picea abies* (L.) Karst.), while mature Scots pines (*Pinus sylvestris* L.) tolerate surface fires (Kolström and Kellomäki 1993, Linder et al. 1998). In addition, after a wildfire, substantial amounts of partly burned tree boles, both standing and lying on the ground, are left on the site; while in prescribed burning the fuel consists of logging residue, ground layer vegetation and the upper parts of the soil organic layer, but no coarse wood is burned. However, the chemical changes in soil brought about by burning are similar regardless of type of burning; only the degree of the changes may depend on the type and intensity of burning.

1.2 Successional stages during burning

Wildfires break out when the moisture content of fuel is low enough for burning and lightning provides the source of ignition, e.g. when a thunderstorm occurs during a summer drought. Lightning provides the external source of heat needed for ignition of the fuel. Whether ignition leads to a larger wildfire is determined by the properties of the fuel, mainly its surface-to-volume ratio, density and moisture content. In general, thin fuels ignite more easily than thick fuels, as the former have a higher proportional capacity to absorb radiation and the mass to be heated is proportionally smaller than that of the latter (Chandler et al. 1983). Low-density fuels reach high surface temperatures and ignite easily because they conduct heat to the interior poorly. Most importantly, moisture affects ignition in several ways; it has to be vaporised before ignition, which consumes energy, and it increases thermal conductivity, which in turn decreases surface temperatures. Depending on the properties of the fuel and on the environmental conditions, ignition may lead to flaming or glowing combustion. Flaming is the process of combustion in which volatiles and extractives expelled from the solid fuel burn in the air. Flaming combustion requires an optimised concentration of flammable hydrocarbons and oxygen. Glowing combustion (or smouldering) occurs when the volatiles have all been expelled, or when the fuel

does not contain flammable volatiles or is unable to outgas them (Johnson 1992). Compared to flaming, glowing is a low-temperature process, and it produces substantial amounts of carbon monoxide (Cofer et al. 1997). In moist low-density fuels it can continue for long periods, and under high winds it can convert to a source of flaming combustion of adjacent unburned material and lead to larger fires (Chandler et al. 1983).

Theoretically, the end products of burning are water vapour, carbon dioxide and mineral elements in the ash. Complete oxidation of biomass requires an optimum input of oxygen during the burning process. However, under natural conditions this is not the case; combustion is incomplete, producing CO, CH_4, H_2, a wide range of hydrocarbons and particulates (Cofer et al. 1997).

1.3 Soil microbes and their environment after fire

1.3.1 Response of soil microbes to fire

One of the first observations of the effect of burning on soil microbes is from the Rubber Research Institute on the Malay peninsula, where virgin forest was felled and the timber burned in heaps (Corbet 1934). This burning increased the numbers of soil bacteria determined as plate counts, but the rise was short-lived and the number of soil bacteria returned to normal very soon after the treatment. The author concluded that the effect was due to increased pH and nutrient concentration, and not related to sterilisation of the soil, since wood ash distributed by wind caused a similar effect on soil bacteria. In contrast to the findings of Corbet, the most frequently reported pattern of response of the total microflora to burning is an immediate decrease in amounts of microbes after the burning. This is followed by a gradual recovery to pre-burn levels or higher, which usually occurs within days (Meiklejohn 1955, Sharma 1981, Deka and Mishra 1983) or months (Ahlgren and Ahlgren 1965, Tiwari and Rai 1977, Theodorou and Bowen 1982, van Reenen et al. 1992). However, there is a wide variety of microbial responses to fire, even in the same ecosystem. First, the response of microbes may be related to the intensity of fire or type of burn. Vázquez et al. (1993) showed increased total numbers of microbes one month after a wildfire in an

Atlantic *Pinus pinaster* forest. In a similar forest, Fonturbel et al. (1995) detected no effects of prescribed burning on the total number of microbes. Second, the effect of fire can also depend on the interval of burning. Hossain et al. (1995) showed that frequent underburning of *Eucalyptus pauciflora* stands (2-3 times a year) reduces microbial biomass, but burning at 7-year intervals increases it.

At best, we can state that in many cases burning reduces microbial biomass temporarily; but generalisations cannot be made, since differences in the reaction of microflora arise from different time of sampling, soil and ecosystem type, intensity of fire and microbiological methodology. The microbial response to fire is often studied using plate-count methods, which may give misleading results, since only a minor proportion of soil microbes is able to grow on nutrient agar (Zuberer 1994) and the percentage of culturable microbes may vary according to changed soil conditions after fire, e.g. higher pH (Bååth and Arnebrant 1994). However, a common observation in previous studies is that burning favours bacteria over fungi (Dunn et al. 1979, Bissett and Parkinson 1980, Sharma 1981, Deka and Mishra 1983).

Since the measured fire-induced response of microbes in field studies cannot be attributed directly to any specific causative effect, a more detailed approach should be chosen for examining the response of the microflora to burning. The factors that function in burning should be recognised and their potential effects on microbes should be investigated one by one. These factors include the direct sterilising effect of heat, formation of ash, charcoal and fire-altered organic matter and modifications of microclimate and vegetation.

1.3.2 Sterilising effect of heat

The initial decrease in numbers of microorganisms commonly observed after burning is caused mainly by the effect of heat liberated in the combustion (Ahlgren and Ahlgren 1965). Although most of the energy released by combustion is lost to the atmosphere, burning causes a more or less severe heat input into the soil, and this heat can immediately kill or injure soil microbes (Walstad et al. 1990). However, the temperature gradient between the flame zone and the mineral soil is usually very steep. While in a study by Swift et al.

(1993) the temperature in the flames reached 800°C, the moist humus layer was heated only to 60°C or, as measured by Vasander and Lindholm (1985), to 30°C. This wide difference between temperatures is explained by the high insulation capacity of the moist humus. First, the organic matter conducts heat poorly; and second, as long as the organic matter is moist, its temperature cannot rise above 100°C. Increasing the moisture content of humus reinforces its insulating capacity (Valette et al. 1994). Thus the moist humus layer prevents the deeper soil from heating up to lethal temperatures. In order to protect the humus layer from complete combustion, clear-cut areas are prescribed burned in the spring when there is sufficient moisture in the humus. In a wildfire, however, the humus layer may be more seriously affected as most wildfires burn during the dry season.

In general, microbes are more resistant to dry heat than to moist heat (Wolf and Skipper 1994), but the susceptibility varies according to type of organism and its life phase (Dunn et al. 1985). Even exposure to 160°C for 3 h was not adequate for complete sterilisation of soil (Labeda et al. 1975). In general, bacteria are more tolerant to heat than fungi are (Bollen 1969). The lethal temperature for bacteria was found to be 210°C in dry soil and 110°C in wet soil, while the corresponding limits for fungi were 155°C and 100°C (Dunn and DeBano 1977). Spores and other resting forms of microbes can tolerate substantially higher temperatures than cells that are undergoing active growth and metabolism.

1.3.3 Changes in soil properties and environment due to fire

Burning of organic matter leaves ash, charcoal and fire-altered material on the ground. Ash contains the inorganic elements that were bound in the vegetation or litter before the fire, except for the proportion which was lost to the atmosphere during burning. An excess of base cations (Ca^{2+}, Mg^{2+} and K^{+}) in the ash leads to increased pH of the soil (Ahlgren and Ahlgren 1960, Raison 1979, Wells et al. 1979). The concentration of mineral nitrogen (NO_3^- and NH_4^+) also shows increased levels after burning, although total nitrogen, which is mainly bound as organic compounds, is partly lost during burning (Kivekäs 1939, Kutiel and Naveh 1987, Little and Ohmann 1988, Gillon and Rapp 1989). The fertilising effect of

burning has been recognised in many studies (e.g. Kivekäs 1939, Austin and Baisinger 1955, Smith and James 1978, Kutiel and Naveh 1987), and therefore burning has been used in agriculture and forestry to improve site productivity. Some authors have linked the increased nutrient concentration to the increased or recovered numbers of microbes (Corbet 1934, Ahlgren and Ahlgren 1965), stating that microbes flourish like plants when they are fertilised by the liberated nutrients. However, in the acid soil of coniferous forests this is obviously not the mode of action, since fast-release fertilisers have been shown to decrease microbial biomass and activity in unburned forest soils (Nohrstedt et al. 1989, Aarnio and Martikainen 1994). Application of slow-release fertiliser (containing P, K, Ca, Mg, S) has been shown not to change either the size or structure of the microbial community (Fritze et al. 1996, Fritze et al. 1997). Accordingly, Salonius (1972) stated that microbial activity in acid forest soils is not limited by N, P or K, but by the availability of decomposable organic matter and the strongly acid conditions. This is verified by the fact that application of ash or lime has been shown to increase microbial activity in unburned, naturally acid soil, which may be caused by increased substrate availability or higher pH (Weber et al. 1985, Zelles et al. 1987, 1990, Bååth and Arnebrant 1994).

In addition to ash, burning of vegetation and other organic matter may produce charcoal as a result of incomplete combustion. The intensity of the fire and the amount of fuel determine the amount of charcoal produced. Since combustion in forest fires is less efficient and the smouldering phase more significant, forest fires produce larger quantities of charcoal than savannah or grassland fires do (Stocks and Kauffman 1997). The beneficial effect of charcoal and its interaction with soil microbes has been demonstrated by Harvey et al. (1976), who showed that charcoal stimulated the formation and activity of ectomycorrhizae. The active role of charcoal as an adsorbing agent in forest ecosystems was revealed by Zackrisson et al. (1996), who showed that a common dwarf shrub, crowberry (*Empetrum hermaphroditum*), in the northern boreal forests in Sweden produces phenolic compounds, which hinder the establishment of Scots pine (Nilsson and Zackrisson 1992). In the absence of charcoal the germination of Scots pine seeds is impaired; but when charcoal is produced by wildfire, it adsorbs the phenolics and contributes to better establishment of Scots pine. In a glasshouse experiment, Wardle et al.

(1998) observed increased substrate-induced respiration under charcoal, which indicates interactions between charcoal and soil microbes.

After a moderate fire, underneath the charcoal layer will be found the humus layer, which has had its chemical composition more or less altered as a result of heating (Jones et al. 1997). The heat-induced changes occurring in forest humus are difficult to determine, as the humus is a complex mixture of compounds, its structure varies and cannot be determined with certainty. Some alterations observed in heated humus are related to the sterilising effect of heat, since the organic substances from the dead microorganisms are liberated in the humus and become available for decomposition by the survivors. In addition to this flush of substrate from the dead organisms, heating itself has been shown to render part of the soil organic matter more soluble (Salonius et al. 1967, Díaz-Raviña et al. 1992) or decomposable (Jenkinson 1966). The mass loss of organic carbon starts between 100°C and 200°C (Sertsu and Sánchez 1978, Kang and Sajjapongse 1980, Giovannini and Lucchesi 1997) due to distillation of the volatile constituents of the organic matter, while above 200°C the organic matter starts to carbonise (Hosking 1938). At 130 - 190°C, lignins and hemicelluloses begin to degrade, and at temperatures under 280°C the adjacent cellulose strands form bonds between each other in a dehydration process (Chandler et al. 1983). Modifications brought about by heating (at 350°C) also include structural changes in humic and fulvic acids and an increase in aromatic structures, which has been proposed to increase the resistance of organic matter to microbial attack (Almendros et al. 1990, 1992, Knicker et al. 1996).

Burning of biomass alters not only the litter and the humus layer, but also the removal of vegetation; in particular, elimination of the shading tree canopy affects the microclimate and evapotranspiration of the site. After a low or moderate intensity wildfire, the microclimate changes less than after prescribed burning of a clear-cut area, since part of the trees usually survive a wildfire. Many of the changes commonly ascribed to prescribed burning are, in fact, caused by the preceding clear-cutting, e.g. removal of trees, dying of tree roots, changes in temperature regimes. Clear-cutting has been shown to affect soil processes, microclimate and composition of plant and animal species (reviewed by Keenan and Kimmins 1993). When the

trees are harvested, both litter fall and root exudation cease. Then, as the sheltering tree canopy is absent, the daytime temperatures tend to increase and night-time temperatures decrease. The throughfall precipitation increases and tree transpiration is missing, but evaporation from the bare and warm surface may compensate for the increase in moisture. Similar changes also occur after wildfires, but they are usually less drastic. These changes evidently affect soil microbes and should be studied separately from the effects of heat, ash or charcoal.

1.4 Special features of fire in boreal coniferous forests

Boreal forests are occupied to a large extent by conifers, which produce needle litter that causes natural acidification of the organic layer. Other typical features of boreal forests are the long winter and short growing season. These factors contribute to a low rate of decomposition and accumulation of organic matter. Thus, the effects of prescribed burning might differ from those observed in more fertile, warmer or deciduous forest ecosystems. There is no general pattern of microbial response to fire, and studies conducted in different ecosystems cannot be directly applied to boreal forests. Thus, the response of soil microbes in the boreal forest humus should be elucidated. In addition, the effect of fires of different intensities should be included in the study; and most importantly, methods that measure the total microbial biomass, not only the culturable proportion in soil, should be used.

Although in many studies the microbial biomass has been shown to recover very soon after burning, the situation in boreal forest soil might differ due to the short growing season and slow turnover of organic matter. The most reliable, although time- consuming, method determining the time required for recovery of the microbial biomass after burning would be to continuously monitor the same burned site. As this is usually not feasible, a more suitable approach would be to select a set of burned sites which vary in time elapsed since burning. Thus it would be possible to ascertain the time required for the microbial biomass to stabilise after burning.

In forest regeneration, clear-cutting is the dominating method of harvesting in boreal forests. The reasons for the changes in microbial biomass after prescribed burning may be caused by clear-cutting, which precedes prescribed burning. To determine to what extent the effects brought about by prescribed burning are actually caused by clear-cutting, prescribed-burned sites should be compared to clear-cut areas and forest stands.

An important feature of boreal coniferous forests that undoubtedly has an effect on post-fire recovery of microbes is the major role of the thick humus layer. Approximately one fourth of the carbon in the soil is found in the humus layer (Liski and Westman 1995), and microbial activity is also highest in that layer. Thus, it can be claimed that the fire-induced changes in the humus layer, as well as the formation of adsorptive charcoal, may be of importance in the reduction and recovery of microbial biomass. Charcoal has the capacity to adsorb organic substances (Zackrisson et al. 1996). The pioneer vegetation of a burned site consists of deciduous tree species, the litter of which releases more water-soluble compounds than that of pine or spruce (Nykvist 1963, Johansson 1995). As charcoal lies between the newly formed litter and the humus layer, charcoal might negatively influence the underlying humus by capturing decomposable substrates from percolating soil water. It was hypothesised, first, that heating of forest humus impairs its properties as a substrate for microbes and second, that charcoal adsorbs decomposable substrates from percolating water, causing substrate deprivation for the microbes inhabiting the humus below the charcoal.

2. Aim of the study

This study concentrates on three consecutive questions. First, the reaction of the soil microbes to prescribed burning and wildfire in the boreal forest was elucidated by measuring the soil microbial biomass, length of the fungal hyphae and respiration activity.

Second, duration of the fire-induced effect on the amount of soil microbial biomass and activity and fungal biomass in a boreal forest was studied using a chronosequence of prescribed- burned sites.

Third, the reasons for the reduction of microbial biomass were elucidated by studying separately the effects of clear-cutting, heat-induced changes in the quality of the humus as a substrate for microbes and the effect of charcoal on the microbes inhabiting the underlying humus.

3. Material and methods

3.1 Field sites

The effect of prescribed burning and wildfire on soil microflora (I) were studied on two field sites, the first one established at the Evo Forestry School in Lammi, southern Finland (61°12'N, 25°7'E) and the second one in Patvinsuo Natural Park in Lieksa, eastern Finland (63°7'N, 30°40'E). In Evo a clear-cut area of 5 ha was prescribed burned in May 1989, and in Patvinsuo a 1.1 ha forest dominated by Scots pine (*Pinus sylvestris*) was set on fire in June 1989 in order to simulate a wildfire. On the clear-cut site in Evo the logging residue was evenly distributed on the site and it was prescribed burned. On the Patvinsuo site the wildfire passed on mainly as a surface fire; only on the windward side did the fire reach the crowns of the trees. At both sites a control forest located adjacent to the burned area. Soil samples were collected at the prescribed-burned and wildfire simulation sites together with their controls twelve and ten times, respectively, during the three subsequent growing seasons from 1989 to 1991.

The duration of fire-induced alterations in soil microbial biomass and activity (II) was studied using a chronosequence of prescribed-burned forest sites regenerated with Scots pine at the Evo Forestry School. The sites (n = 18) were located within an area of *ca* 12 km^2 and the time elapsed since burning ranged from 0 to 45 years. The sites were sampled on two occasions (in June and August) during the 1991 growing season.

The effect of initial clear-cutting before burning (III) was studied in Suomussalmi, northern Finland (65°15'N, 28°50'E), where clear-cutting with or without prescribed burning was conducted on 50 m x 50 m plots in a random block design with four blocks, using plots of uncut mixed stands of Norway spruce (*Picea abies*) and Scots pine as controls. The samples were collected in June 1993, when three years had elapsed since cutting and two years since burning.

3.2 Microcosm studies

Two microcosm experiments were conducted in the laboratory. The alterations in humus quality and its capacity to support microbial life were studied using dried and heated forest humus. After drying at 45°C, the humus was divided into portions, each with a mass of 25 g. The samples were subjected to seven different temperature treatments: 60°C, 80°C, 100°C, 120°C, 140°C, 160°C and 230°C. The control samples had experienced only the temperature during drying (45°C). After heating, the humus samples were moistened and simultaneously inoculated with original, untreated humus suspension. The samples (8 treatments x 4 samplings x 4 replicates = total number of 128 samples) were incubated at 14°C in the dark, and sampled after 1, 2, 4 and 6 months.

The adsorbing capacity of charcoal and its possible suppressing effect on the underlying untreated humus (V) was studied using two-layered microcosms with a covering layer of one of the adsorbents; 1) pumice as negative control (Pum), 2) charcoal prepared from *Empetrum nigrum* twigs (EmpCh), 3) charcoal prepared from forest humus (HuCh) or 4) commercially manufactured activated carbon (ActC), with an underlying layer of forest humus. The microcosms were moistened regularly with extract of birch leaf litter, which

contained easily decomposable carbon sources. After 29 days the microcosms (4 treatments x 4 replicates = 16 microcosms) were sampled, and the adsorbents and humus were treated separately (number of samples = 32).

3.3 Microbiological analyses

3.3.1 Microbial biomass

In Studies I, II and III, soil microbial biomass was measured by the fumigation-extraction method (FE) (Brookes et al. 1985, Vance et al. 1987) The fumigation method measures the microbial cells which have been lysed by gaseous chloroform. After fumigation of the soil samples, the carbon liberated from the cells was extracted and measured. Extractable carbon was calculated as soil microbial biomass carbon (C_{mic}; μg C g^{-1} soil d.m.) using an equation derived from Martikainen and Palojärvi (1990): C_{mic} = C from fumigated sample - C from non-fumigated sample) · 1.9 + 428. In Studies III, IV and V, the substrate-induced respiration (SIR) measurement, as described by Priha and Smolander (1994) and based on the theory of Anderson and Domsch (1978), was used for assessing C_{mic}. The SIR method takes into account the metabolically active and aerobic proportion of microbial biomass, which is able to form CO_2 from glucose.

Fungi were determined by measuring the length of the fungal hyphae under the microscope (I) after the diluted soil sample was filtered on a 0.8 μm membrane filter (Hanssen et al. 1974, Sundman and Sivelä 1978, Fritze et al. 1989) or by extracting the fungal ergosterol directly from the soil (II, III) (Grant and West 1986). In most fungi ergosterol is the predominant sterol (Tunlid and White 1992) and the amount has been shown to correlate with the fungal surface area (West et al. 1987).

3.3.2 Activity and growth

The respiration activity of the microbial biomass was measured using the static method, i.e. sealed chambers, and sieved soil. The CO_2 production at 14°C was measured by gas chromatography using field-

moist (I,III) or moisture-adjusted soil samples (I, II, III, V) (Zibilske 1994).

For assessment of nitrogen mineralisation activity and nitrification, moisture-adjusted soil samples were incubated at 14°C; and the amounts of NH_4^+-N and NO_3^--N were determined from incubated and control samples prior to incubation and after 6 weeks (III).

The *in situ* rate of litter decomposition was studied on the prescribed burned site and on the wildfire-simulation site using dried Norway spruce or Scots pine needles, respectively, placed in the soil organic layer in a bag of polyethene fabric (I). The litterbags were stored in the humus for one and two years.

The ^{3}H-thymidine incorporation technique was used to measure the growth rate of bacteria released from the humus and the adsorbents (V) (Bååth 1992, Pennanen et al. 1998). The bacterial suspension for the assay was prepared by adding humus or adsorbent to water. The suspension was shaken and centrifuged, and the supernatant was filtered through quartz wool. A portion of the suspension was incubated for 2 h with ^{3}H-thymidine. The labelled thymidine incorporated into the bacterial cells (total macromolecules) was measured with a liquid scintillation counter and the ^{3}H-thymidine incorporation was calculated as mol TdR g^{-1} d.m. h^{-1}. After staining with acridine orange, the bacterial cells in the incubation suspension were counted.

3.3.3 Community profiling

The phospholipid fatty acids (PLFA) present in the cell membranes of the soil microbes were determined as described by Frostegård et al. (1993) (IV, V). The individual PLFAs were expressed as percentage of the total amount of PLFAs detected in a soil sample. Using nonadecanoate (19:0) as an internal standard, the total amount of PLFAs in the soil sample was calculated (Frostegård et al. 1993). This value was used as an indicator of soil microbial biomass (IV), since PLFAs occur universally in cell membranes and are not storage compounds (Frostegård et al. 1991). Twelve PLFAs (i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, 17:0, cy17:0, 18:1ω7 and cy19:0) were taken to represent bacterial origin, and one (18:2ω6,9)

was used to represent fungal biomass (Frostegård and Bååth 1996).

The commercially manufactured Biolog® Ecoplates, containing 31 different substrates (listed by Insam 1997; note that α-keto glutaric acid should be α-keto butyric acid), and water, serving as control, were used to assess the potential substrate utilisation capacity of microbes (IV, V). The wells of the Ecoplates were filled with *ca* 10^{-3} diluted sample-water suspension and incubated at 20°C for seven days. The absorbance values (at 590 nm) of the subsequent readings were corrected for background absorbance by subtracting the value for the water well, and the area under the absorbance curve was calculated (Sharma et al. 1997) for each substrate. The substrate utilisation efficiency was expressed as percentage of individual substrates from the sum of all areas under the curve.

3.4 Physico-chemical analyses

The percentage of dry matter in a sample was determined after samples were dried at 105°C overnight. Proportion of organic matter was calculated as the percent of loss-on-ignition (at 550°C for a minimum of 3 h) from soil dry matter (Howard and Howard 1990). The pH of the samples was measured from soil:water suspensions. Total nitrogen was determined by the Kjeldahl method (I) (Halonen et al. 1983, Bremner 1996) or with a LECO CHN analyser (Nelson and Sommers 1996). For determinations of the exchangeable nutrients Ca, K, Mg and Na, the humus samples were extracted with acidic ammonium acetate (I) or $BaCl_2$ (III), and concentrations of elements were measured on an atomic absorption spectrophotometer (I) or an inductively coupled plasma emission spectrometer (III). Exchangeable acidity of the humus was measured by titrating the $BaCl_2$ suspension with NaOH to pH 7 (III).

The near infrared reflectance (NIR) spectra were measured on freeze-dried and homogenised humus samples (III) (Palmborg and Nordgren 1993). The Fourier-transform infrared (FTIR) spectra accomplished by ^{13}C-NMR spectra were measured on air-dried and mortared humus samples (IV). The interpretation of the FTIR spectra was based on data by Stevenson and Goh (1971), Holmgren and Nordén (1988), Lin-Vien et al. (1991) and Celi et al. (1997). The

NMR spectra were divided into seven regions representing different carbon types according to data by Malcolm (1990) and Barančíková et al. (1997).

3.5 Statistical analyses

Differences between treatments were detected using ANOVA, followed by Tukey's test. When the assumptions of normal distribution or equality of variances were not met, the results were log transformed or Kruskall-Wallis non-metric ANOVA was used. Principal component analysis (PCA) was used to reduce the number of variables and concentrate the variation observed in the data into principal components. PCA was performed on all measured variables (I), mole percentage values of PLFAs (IV, V), area percentage values for substrates of Biolog-plates (V) or transmittance values of NIR scanning (III). When the scores for the sample points were plotted on the principal components, the possible separation of the treatments could be detected on the biplot. The separation of treatments was tested by subjecting the sample scores of the principal components to ANOVA, followed by Tukey's test (IV, V). Canonical discriminant analysis was used to detect the separation of the differently heated humus samples on the basis of their substrate utilisation capacity (IV). The partial least squares (PLS) analysis was applied to the biological data (basal respiration activity, amount of ergosterol, C_{mic} by SIR and C_{mic} by FE) and the NIR transmittance data measured at the clear-cut, the clear-cut + burned and the control sites at Suomussalmi (III). The biological data were read, one variable at a time, into the Y-matrix and the NIR data into the X-matrix. The PLS then uses these two matrices simultaneously to determine the regression model between the main effects observed in the matrix data (The Unscrambler 1996).

4. Results and discussion

4.1 Microbial biomass and activity, and soil chemical properties after prescribed burning and wildfire

Both prescribed burning after clear-cutting and wildfire reduced the amount of microbial biomass carbon (C_{mic}) in the humus layer (I). During the first growing season the reduction was 35% and 16% of the control level for prescribed burning and wildfire, respectively (Fig. 1), when calculated using values of C_{mic} per organic matter (o.m.).

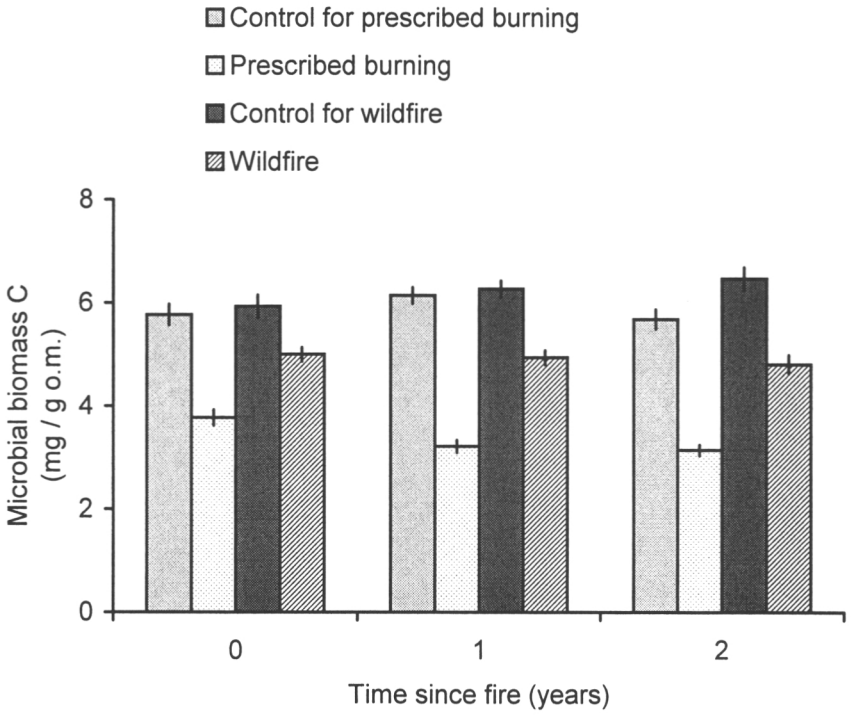

Fig. 1. Microbial biomass carbon measured by fumigation-extraction method and expressed per amount of humus organic matter as annual mean values of the prescribed burned, wildfire simulation and their control sites during two years after the fire. Error bars represent the standard error of the mean.

The fire intensity, i.e. the rate of heat released per length of fire front, in kW m^{-1} (see e.g. Johnson 1992), of these burns was not measured; but the severity of the fire was described on the basis of the amount of fuel consumed and the extent of changes caused by the fire. Since calcium has a high boiling point (1484°C) in relation to the commonly observed flame temperatures, losses of Ca during burning are limited mainly to losses in particulate form in the ash. Compared to other major nutrients, losses of Ca due to burning have been shown to be the smallest (Harwood and Jackson 1975, Raison et al. 1985, Gillon and Rapp 1989). Since a major part of the Ca formerly present in the burned biomass is concentrated in the ash and humus layer, the increase in amount of Ca can be used to estimate the fire severity. As the concentration of Ca after prescribed burning increased 3-fold but after wildfire simulation only 1.5-fold (calculated using kg ha^{-1} values), it can be inferred that the prescribed burning was a more severe treatment than the wildfire simulation. The higher severity was also confirmed by the fact that the flames were higher during the prescribed burning, and it induced greater changes in vegetation than the wildfire did (personal observation).

As for Ca, the concentrations of Mg and K increased after prescribed burning and wildfire; but after the first growing season, later K was obviously lost through leaching. These changes in extractable nutrients were accompanied by an increase in the pH value, which, compared to the control sites, was 2.1 and 0.5 pH units higher on the prescribed-burned and wildfire sites, respectively. All the changes in nutrient levels and pH followed the same trend but, compared to the prescribed-burned site, were much smaller on the wildfire simulation site.

The total amount of C_{mic} was reduced more at the prescribed-burned site, and the amount remained below the control level throughout the three growing seasons after the fire. The length of the fungal hyphae was also reduced consistently. The wildfire caused a smaller reduction in C_{mic} and in the length of fungal hyphae than prescribed burning did. The respiration activity measured in field moist samples varied between individual samplings more than C_{mic} did, because the respiration activity depends strongly on the prevailing moisture and weather conditions during sampling. The lower water content of the prescribed-burned humus samples was obviously one

reason for the low level of basal respiration in these samples, especially during the third growing season after the fire (I, Fig. 4 E), since very dry or very wet conditions lead to reduced soil respiration (Bowden et al. 1998, Gulledge and Schimel 1998). When basal respiration was measured using moisture-adjusted humus, the respiration activity in humus from the prescribed- burned site was at least three times higher than that measured in field moist humus, but still remained lower than the control. Reduction in soil respiration after clear-cutting and burning has also been shown in an aspen ecosystem (Weber 1990). However, the difference in basal respiration between the wildfire site and the control was smaller, and the wildfire site did not respond to addition of moisture. These results indicate that the microbes of the prescribed-burned humus were under moisture stress.

The ratio of basal respiration (as measured with field moist soil) to microbial biomass carbon, i.e. the specific respiration of the biomass (qCO_2), was clearly higher on the prescribed-burned site than in the control for the first growing season after the fire. Similarly, the qCO_2 was higher on the wildfire site than in the control, but the difference was smaller. Disturbances, i.e. rapidly changing environmental conditions, are known to cause increased values of qCO_2, also qCO_2 was been shown to decline during ecosystem development during succession (Wardle and Ghani 1995). However, a strong negative relationship has been shown to exist between qCO_2 and the amount of microbial biomass, indicating that factors which cause reduced microbial biomass enhance the specific respiration activity (Šantrůčková and Straškraba 1991, Wardle and Ghani 1995). Obviously the disturbance in the form of fire and the resulting smaller amount of microbial biomass increased the qCO_2 on both burned sites.

Regardless of the reduced basal respiration activity, the rate of *in situ* litter decomposition rate (measured as mass loss from litter bags) on the prescribed burned site was faster than in the control. On the contrary, litter decomposition in the humus of the wildfire site was slower than that in its control (I). The differential rate of litter decomposition on the prescribed- burned and wildfire sites may be caused by differences in nutrient concentration of the humus or temperature regime. The microbes decomposing the needles in the litter bags need nutrients either from the substrate or from the soil

solution (Aber and Melillo 1991). Addition of nitrogen has been shown to accelerate the first stage of needle-litter decomposition, especially degradation of cellulose (Mikola 1954). In the later stage of decomposition, when lignin and lignified celluloses are the major compounds in the litter, high levels of nitrogen retard decompositition by inactivating the lignolytic enzyme system (Berg 1986). On the prescribed-burned site the amount of extractable nitrogen in humus during the first growing season after the fire was almost ten times that in the humus of the wildfire site. As a result, the high level of extractable nitrogen on the prescribed burned site may have accelerated the first stage of litter decomposition. The difference levelled off in the later stage of decomposition in the second growing season (2-year litterbags). Accelerated litter decomposition was not observed on the wildfire site, which is in accordance with the lower level of extractable N. In addition, the higher daytime temperatures on the treeless prescribed-burned site may have accelerated litter decomposition compared to the wildfire site, where mature Scots pines survived the fire.

Obviously the reduced C_{mic} and shorter length of fungal hyphae caused by the prescribed burning were partly related to the previous clear-cutting, which altered the species composition of plants. The wildfire simulation affected the plant community less, since a number of the mature Scots pines survived the fire, and the post-burn field layer vegetation consisted mainly of forest species regenerating from underground reproductive organs, compared to the prescribed-burned site where the composition of the vegetation changed completely and consisted of pioneer species, e.g. *Calamagrostis* grasses, *Epilobium angustifolium* and *Rubus saxatilis* (personal observation). The harvesting of trees may also have accounted for some of the reduction in the length of fungal hyphae, as the cessation of carbon allocation to the roots caused by clear-cutting is obviously related to the decrease of fungal hyphae in forest soil (Bååth 1980).

In conclusion, both types of fires reduced the amount of microbial biomass in the humus, and during the three-year study period the microbial biomass did not recover to the control level. Prescribed burning caused a sharper reduction in microbial biomass and activity, which was obviously due to its greater severity, wider changes in soil chemistry and elimination of trees.

4.2 Stabilisation of microbial biomass and activity after prescribed burning

From Study I it was clear that three years was not adequate for recovery of microbial biomass and activity after burning. Therefore, the time needed for stabilisation of the microbial biomass was studied using a chronosequence of prescribed burned sites ranging in age from 0 to 45 years (II). On the sites which had been burned less than 9 years ago the amount of C_{mic} was substantially lower (2.8 - 4.4 mg g^{-1} o.m.) than on the sites with longer (12 - 45 years) succession after fire (5.6 - 8.0 mg g^{-1} o.m.). An exponential function [$y = a(1- e^{-bt}) + c$, where t is time since beginning of succession], which has been shown to best describe the changes in C_{mic} in old fields of increasing age (Zak et al. 1990), was fitted to the measured C_{mic} values (Fig. 2). The function provided a satisfactory fit with $R^2 = 0.602$.

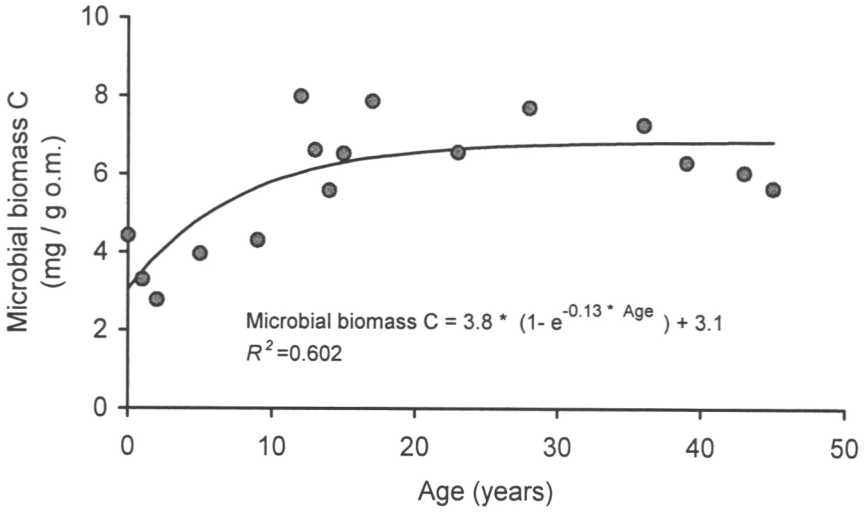

Fig. 2. Microbial biomass carbon as measured by fumigation-extraction method and calculated per amount of humus organic matter for a series of prescribed-burned sites ranging in age from 0 to 45 years after burning. The fitted curve represents the exponential model y = a(1 - e^{-bt}) + c. The parameters and regression coefficient are given on the figure.

The deviation of the 0-, 1- and 2-year-old sites is probably caused by site-specific variation, since a comparable drop in C_{mic} 1 or 2 years after burning was not observed during the three-year monitoring of the prescribed-burned site at Evo (I). The levelling of the predicted values for C_{mic} occurred *ca* 15 years after the fire. The level of C_{mic} reached after 15 years corresponds to measurements of C_{mic} in the control forest stands in Study I (5-8 mg g^{-1} o.m.). Unfortunately, the sequence of prescribed-burned sites did not contain replicate sites, but this would have improved fitting of the curve.

Total microbial biomass (sensitive to chloroform fumigation), soil basal respiration and amount of ergosterol gave consistent results along the chronosequence. Fungal biomass, as estimated by ergosterol content, was low (100-170 μg g^{-1} o.m.) during 0-9 years after burning, but on the older sites increased to 200-300 μg g^{-1} o.m. The specific respiration of the microbial biomass, i.e qCO_2, was higher immediately after the fire, but then in the third growing season it declined to a stabilised level. The trend in qCO_2 may reflect the initially low amount of microbial biomass, which later stabilised, as discussed in the previous section. However, the time needed for the stabilisation of the qCO_2 was only 2-3 years compared to the clearly longer time (15 years) needed for the stabilisation of the microbial biomass. The drop in all measured variables on 43- and 45- year-old sites, which was seen most clearly for soil basal respiration (II, Fig. 1), may have been caused by the thinning and fertilising practices used on the oldest sites.

The time needed for stabilisation of C_{mic} was *ca* 15 years, which, as discussed in the introduction, is substantially longer than reported for many other ecosystems. A recovery time comparable to the estimated 15 years has been reported by Chang and Trofymow (1996), who measured the lowest microbial biomass in three-year-old stands of *Thuja plicata - Tsuga heterophylla* in British Columbia, Canada, but showed that 10-year-old stands of similar vegetation were recovering to the levels found in an old-growth forest. As reported by Prieto-Fernández et al. (1998), the recovery time after wildfire in a *Pinus pinaster - P.silvestris - P.radiata* ecosystem in northwestern Spain was somewhat shorter. They showed that on sites ranging in age from 1 day to 4 years after wildfire the microbial biomass was, on average, 60% of that on unburned sites; and four years after the fire the

difference between burned and unburned plots was reduced.

4.3 Reasons for the reduction in microbial biomass and its slow recovery

4.3.1 Clear-cut harvesting

From the comparison between prescribed burning and wildfire, it was obvious that the former induced a more profound change in humus material and among the microbes inhabiting the humus, and that the difference may have been partly due to the effect of clear-cutting. Since the decline in C_{mic} observed after prescribed burning might be caused by the preceding clear-cutting, a clear-cut site was compared to a clear-cut and burned site, with a uncut forest stand as control. In the fourth growing season after clear-cutting C_{mic} (calculated per amount of organic matter in humus) was 26% and 20% lower than in the control, when C_{mic} was measured by FE and SIR, respectively (III). The C_{mic} might have peaked soon after clear-cutting, but at the time of sampling it was under the control level. The prescribed burning of the clear-cut plots resulted in a significant and even larger decrease in C_{mic} compared to clear-cutting alone. As measured by FE and SIR, the reduction compared to the control was 60% and 42%, respectively. The amount of ergosterol was reduced by 31% on the clear-cut site and by 63% on the clear-cut + burned site compared to the control. The more pronounced reduction in amount of ergosterol than in total biomass, especially after clear-cutting, might indicate that the fungi suffered more from the treatments than bacteria did. Bååth et al. (1995) confirmed this by showing that the ratio of fungal to bacterial PLFAs decreased after clear-cutting and even more after prescribed burning. Often the amount of soil bacteria peaks after tree harvesting (Sundman et al. 1978, Lundgren 1982), but the amount of fungi declines (Bååth 1980). The effect of clear-cutting on total microbial biomass can be negative (Bauhus and Barthel 1995), negligible (Smolander et al. 1998) or positive (Entry et al. 1986). The response of the total biomass is obviously related to the relative proportions of fungi and bacteria prior to clear-cutting, as clear-cutting increases the ratio of bacteria to fungi (Entry et al. 1986, Bååth et al. 1995). Thus, in a fungal dominated soil the total microbial biomass is affected more severely.

As a result, the total reduction in C_{mic} was not merely caused by the preceding clear-cutting, since the reduction caused by clear-cutting alone was only a part of the reduction caused by the combination of the treatments. The minor effect of clear-cutting compared to burning was also supported by the findings of Fritze et al. (1994), who measured drastically reduced values for microbial biomass after experimental underburning of an uncut forest stand, where the mature Scots pines on the site were not affected by the fire. In conclusion, clear-cutting only partly explained the reduction in microbial biomass, which means that the major reduction is due to burning, either directly or via introduced environmental changes.

4.3.2 Fire-induced changes in the properties of humus

At Suomussalmi, the amount of extractable NH_4^+-N was significantly higher on both clear-cut and clear-cut + burned sites (190-200 μg g^{-1} o.m.) than on the control site (52 μg g^{-1} o.m.). When the humus samples were incubated at 14°C, in the humus from a clear-cut + burned site, during a 6-week laboratory incubation, the amount of NO_3^--N increased from 19 to 118 μg g^{-1} o.m. (III, calculated using data from Tables 1 and 3). In humus from clear-cut and control sites, nitrate was not formed during the incubation. Obviously the increased pH together with the higher NH_4^+-N concentration accelerated nitrification, since these factors have been shown to stimulate nitrification in acid soils (Martikainen 1984). In the chronosequence of sites from 0 to 45 years after burning, for two years after burning the pH of the humus remained above 5, and nine years after burning it had reached the background level (II). When these same humus samples (0 - 45 years) were incubated for 28 days at +14°C, net formation of NO_3^- was detected only in the humus from sites on which 0, 1 or 2 years had elapsed since the fire (Pietikäinen and Fritze 1996). Thus, the high pH for two years after burning was obviously linked to nitrification activity. In addition, the reduced competition by heterotrophs after soil heating may contribute to the higher activity of autotrophic nitrifiers (Bauhus et al. 1993).

The near infrared reflectance (NIR) spectra of the humus from the clear-cut, clear-cut + burned and control sites were measured; and the transmittance values were subjected to PCA, which clearly separated

the clear-cut + burned site from the two other sites (III, Fig. 2). Palmborg and Nordgren (1993) showed that the NIR spectra of organic matter composition can be used to model the variation in soil basal respiration and SIR. When a PLS regression between the NIR data and the microbiological variables [1) basal respiration, 2) amount of ergosterol, 3) C_{mic} determined by SIR and 4) C_{mic} determined by FE] of the clear-cut, clear-cut + burned and control humus samples were calculated, the NIR data explained 62%, 79%, 75% and 82% of the variation in the microbiological variables, respectively. As a result, it can be concluded that the fire-induced changes in humus composition as measured by NIR could be used to determine microbial activity and biomass after burning.

As these humus samples, in which the NIR measurements showed fire-induced structural changes, consisted of an undefined mixture of partially charred organic matter, charcoal, ash and most of all, unburned, but to some extent heated humus, it was found necessary to study the effect of heating on the properties of humus under controlled laboratory conditions, where the formation of ash and charcoal could be eliminated. Collecting heated humus in the field is not feasible as the actual temperature during the burning should be known for every sample. In addition, the moisture contained naturally in the humus causes a vertical temperature gradient in humus as it evaporates. In order to determine the actual temperature experienced by every sample, the humus was heated in an oven. Air-dried forest humus was heated in thin layers to reduce evaporation of water, and to decrease the vertical temperature gradient (IV).

Heating at 45 - 160°C did not change the visual appearance, percentage of organic matter or carbon-to-nitrogen ratio of the humus. However, heating at 160°C decreased the pH of humus by 0.5 units. A similar decreasing effect of mild heating on pH has been reported by Kitur and Frye (1983), Saarinen (1989) and Nishita and Haug (1972), who suggested that the decreased pH might be due to organic acids released in the soil. The microflora established equally well in the 140°C- and 160°C-heated samples and the control (heated at 45°C), when the samples were moistened, inoculated and incubated at 14°C, while somewhat lower amounts of C_{mic} were found in humus heated at 100°C. However, the C_{mic} in the 140°C- and 160°C-heated humus decreased throughout the incubation (1-6 months). After six

months the C_{mic} level of the 45 - 230°C -treated samples was negatively related to the heating temperature (IV, Fig. 2). The humus sample heated at 230°C was partially charred and had a clearly higher pH (5.4) than the control (4.3). It differed from all the others by showing poor establishment of microflora from the beginning of the incubation. Six months after inoculation of the samples the lowest C_{mic} was found in the 230°C-treated sample (1.1 mg g^{-1} o.m.), which was 30% of the respective control. Similar results have been reported using samples with both low (mineral soil) and high (wood) organic matter content. Díaz-Raviña et al. (1992) heated aliquots of humic cambisol (C content 7.1%) at 160°C, 350°C or 600°C for 30 min and found that, although the soils were inoculated prior to incubation, the degree of microbial recovery depended on the heating temperature. They showed that after a two-week incubation, C_{mic} (measured by FE) was 50%, 7% and ~0% of the control in the samples heated at 160°C, 350°C and 600°C, respectively. Accordingly, they suggested that soil conditions for recolonization were poorer after intense heating. Baldock (1999) confirmed that heating also reduced the microbial degradation of wood. He showed that after heating at temperatures over 200°C little carbon was available to microorganisms when the heated wood samples were inoculated and incubated for 120 days, and that the decreased bioavailability of the wood was related to a conversion from carbohydrate carbon to aromatic C.

Not only was the size of the microbial biomass affected by the dry heating of humus, but the structure of the microbial community established in the differently heated humus samples, as assessed by extracting the microbial phospholipid fatty acids from the humus, also differed significantly (IV, Fig. 4). The microbes inhabiting the humus treated at 45 - 100°C were characterised by a different set of PLFAs than those microbes inhabiting the humus treated at 120 - 160°C. The differently heated humus samples were also separated by characterising the substrate utilisation patterns of the microbes using Biolog microplates. Accordingly, dry heating resulted in altered microbial community structure and changes in the properties of humus as a habitat and substrate for microbes. Shifts in the wave numbers of the NIR spectra measured from the 230°C-treated humus samples indicated increased aromatic properties. This is in accordance with the findings of Knicker et al. (1996), who revealed, by measuring ^{13}C-NMR spectra of heated plant biomass, that the carbohydrate fraction

was converted to dehydrated material with aromatic properties. In the humus samples heated at temperatures from 45°C to 160°C, no differences in the composition of humus were detected either in the FTIR or NMR spectra, although the microbial community structure and the substrate utilisation pattern differed between humus samples treated with mild or severe heat. However, these changes would probably occur in a small fraction of the humus, and thus neither FTIR nor NMR, both of which measure the composition of the whole humus layer, was able to detect such small alterations.

The reduction in the size of microbial biomass in the 160°C-treated humus from one month to six months was accompanied by an increasing ratio of *trans* to *cis* configurations of PLFA 16:1ω7 i.e. the *t/c* ratio (IV, Fig. 5). The *t/c* ratio is a proposed stress index, which has been shown to increase in microbes in a pure culture due to starvation, desiccation or osmotic stress (Guckert et al. 1986, Kieft et al. 1994, Heipieper et al. 1996). The increase in *t/c* ratio in the microbes of the 160°C-treated humus might indicate a flush of an easily decomposable carbon source initially after heating and inoculation, which during the prolonged incubation would later be depleted and thus cause starvation and reductions in number of established microbes. The proposed initial flush of easily decomposable carbon sources is in accordance with the results of Serrasolsas and Khanna (1995), who observed a period of high respiration 0 - 30 days after heating of the soil and a subsequent decrease during the second phase: 30 - 210 d. However, the use of the *t/c* ratio as a stress indicator for mixed cultures (like in a soil sample) can be questioned, as the changes in the ratio could also arise from changes in species composition during incubation. In a mixed culture, like the microbes in this study, the changes in *t/c* cannot be attributed solely to cell-specific or species-specific changes. Temperatures similar to those used in this laboratory experiment can also be encountered in field conditions. If the humus was dry before fire, the situation would be equivalent to heating of dry humus in an oven. However, when the humus is moist, the heat-induced changes may be more severe, as the action of water accelerates the reactions in humus (Salonius et al. 1967).

A factor that may additionally reduce microbial establishment after burning might be the fire-induced formation of inhibitory compounds

in the soil. An aqueous solution extracted from burned or heated soil has been shown to inhibit fungi (Widden and Parkinson 1975), bacteria (Díaz-Raviña et al. 1996) and soil respiration (Fritze et al. 1998). However, the inhibitory substances have not been purified or identified, and they are not present on all burned sites (J. Pietikäinen, unpublished results).

Since heating of the humus resulted in a changed microbial community, it can be proposed that some of the microbes favour heated substrates and might even be specialised in decomposing fire-altered humus or wood. From the standpoint of retaining microbial diversity, it would be beneficial to study the microbial decomposition and decomposer food-webs in scorched wood, especially logs and snags that have been abundant in the boreal ecosystem before the human impact (Linder and Östlund 1998). As many insects and fungi require or favour burned wood (Muona and Rutanen 1994, Wikars 1997), other specialised organisms and symbiotic relationships might also be found in burned wood or fire-altered soil organic matter.

4.3.3 Charcoal

The adsorbing capacity of charcoal was studied in a laboratory experiment in which microcosms with an overlying adsorbent and an underlying humus layer were watered with birch leaf litter extract (V). The litter extract contained 170 mg l^{-1} glucose, which was included in the total concentration of organic C (730 mg l^{-1}). The adsorbents bound organic compounds with different affinities; the adsorbing capacity increased in the order pumice (Pum) < charcoal from humus (HuCh) < charcoal from *Empetrum nigrum* twigs (EmpCh) < activated carbon (ActC). If the charcoals and ActC had during the one-month incubation adsorbed organic substrates to a substantially greater extent, the microbial biomass under them should have been smaller than that under pumice, which does not have adsorbing capacity. This was not confirmed in the study, since the effect of charcoal (and activated carbon) on the total microbial biomass of the underlying humus was found to be negligible. The only effect of charcoal on the humus was obviously induced via a pH effect: two of the adsorbents, EmpCh and ActC, increased the pH of the underlying humus, which

was reflected in the increased rate of basal respiration in these humus samples (V, Fig. 1). It was concluded that neither the charcoal nor its indirect effects were responsible for the commonly observed reduced amount of C_{mic} after burning.

Surprisingly, the ecological significance of charcoal lay in the fact that the charcoal itself supported a microbial community which was small but more active than that of the humus. When all the adsorbents were provided with similar environmental conditions and substrate, it was found that the size and structure of the microbial community depended on the properties of the adsorbent. After the one-month incubation, the size of the microbial biomass in the adsorbents followed the order EmpCh > HuCh > ActC > Pum (V, Fig. 1). Activity, measured as basal respiration and rate of bacterial growth rate, were higher in both charcoals than in ActC or Pum. The specific growth rate, i.e. growth per bacterial cell, did not differ significantly between adsorbents, although the microbial communities established in the adsorbents differed with respect to their PLFA and their substrate utilisation patterns. Microbial communities of ActC and EmpCh resembled each other with regard to their PLFA patterns, while the community in Pum clearly differed from the first group (V, Fig. 3). Obviously, the microbes attached themselves to charcoal (or activated carbon) particles and degraded the adsorbed substrates like in biological activated carbon beds (De Laat et al. 1985, Kim et al. 1997). In conclusion, when moistened with substrate-rich litter extract, the charcoal formed by combustion was capable of supporting microbial communities.

The results of this microcosm study indicate that charcoal has the potential to support microbial communities. However, this does not imply that the same would also be true in the field; but the presence of microbial communities in the charcoal layer should be studied after a fire in natural conditions. Conditions similar to those simulated in the microcosms might be expected to occur from two years after burning onwards, when pioneer vegetation has covered the burned area and produces abundant litter. Charcoal might also be favoured by soil microbes because, as suggested by Zackrisson et al. (1996), the micropore structure of charcoal could shelter microbes against predators. Usually the charcoal layer is discarded when samples are collected after fire, but it might be more beneficial to include this

layer in the study as a separate sample.

4.3.4 Other reasons

The sterilising effect of heat, as discussed in the introduction, was presumed to be short-lived and to be restricted only to the upper layers of soil. Thus it was not regarded as a probable reason for the long-term reduction in microbial biomass found in this study. As lethal temperatures seldom reach the mineral soil, there are always microbial survivors in the deeper soil layers. Jalaluddin (1969) showed that, due to invasion of mycelia from surrounding soil, fungal colonisation of a small circular burned area was most pronounced at the margins; in addition, fungi originating from wind-dispersed spores were isolated from the centre of the burns. As stated by Finlay et al. (1997), microbes are extremely abundant and easily dispersed, so new microbial niches are likely to be filled within a short time.

Changed vegetation and microclimate have been shown to affect soil microbes (reviewed by Wardle 1992). Burning changes the species composition of vascular plants and mosses (Nykvist 1997) and almost eliminates the litter crop of trees, which may be 1000 - 2600 kg ha^{-1} in a mature Scots pine or Norway spruce stand (Viro 1955, Bonnevie-Svendsen and Gjems 1957, Mälkönen 1974). The decreased litter input from trees is partly compensated for by the high biomass of the field layer vegetation, which may be 1600 - 3000 kg ha^{-1} after clear-cutting or clear-cutting followed by burning (Campbell et al. 1977, Nykvist 1997, Slaughter et al. 1998). However, in this study, although the importance of changed vegetation and climate is significant, these effects could not be separated from the overall effects of clear-cutting.

5. Conclusions

A simulated wildfire of low severity spreading mainly as a surface fire affected the microbial and physico-chemical properties of the humus layer less than standard prescribed burning of a clear-cut forest stand where stems had been harvested but branches and needles left to burn. The prescribed fire and wildfire reduced the amount of C_{mic} by 35% and 16%, respectively. The decline in amount of C_{mic} caused by prescribed burning stabilised in *ca* 15 years to levels commonly found in unburned humus (5 - 8 mg C_{mic} g^{-1} o.m.).

Clear-cutting alone reduced microbial biomass less (20-26%) than clear-cutting followed by prescribed burning (42-69%). When dry humus was heated at 230°C and partially charred, changes in the structure of the humus were detectable by FTIR spectroscopy, the pH of the humus increased significantly, the amount of microbial biomass in the humus remained low and the structure of the microbial community differed from that of the control. Heating at lower temperatures (120-160°C) below the ignition point also caused reduced microbial biomass over the long term and changed the microbial community structure, although no differences in the structure of humus could be detected by FTIR or ^{13}C-NMR spectroscopy. Charcoal did not reduce the size of the microbial biomass in the underlying humus, but supported the microbial communities itself. The microbial community in charcoal was small, but had a higher specific growth rate than that in humus did. The reduced size of the microbial biomass observed after burning was at least partly caused by clear-cutting and partly by the fire- or heat-induced changes in the humus.

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Paper I

I

Pietikäinen, J. and Fritze, H. 1993. Microbial biomass and activity in the humus layer following burning: short-term effects of two different fires. *Canadian Journal of Forest Research* 23: 1275-1285.

Microbial biomass and activity in the humus layer following burning: short-term effects of two different fires

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During a 3-year study, soil microbial biomass C and N, length of the fungal hyphae, soil respiration, and the percent mass loss of needle litter were recorded in coniferous forest soil humus layers following a prescribed burning (PB) treatment or a forest fire simulation (FF) treatment (five plots per treatment). Unburned humus from adjacent plots served as controls (PC and FC, respectively). Prescribed burning was more intensive than the forest fire, and this was reflected in all the measurements taken. The amounts of microbial biomass C and N, length of fungal hyphae, and soil respiration in the PB area did not recover to their controls levels, whereas unchanged microbial biomass N and recovery of the length of the fungal hyphae to control levels were observed in the FF area. The mean microbial C/N ratio was approximately 7 in all the areas, which reflected the C/N ratio of the soil microbial community. Deviation from this mean value, as observed during the first three samplings from the PB area (3, 18, and 35 days after fire treatment), suggested a change in the composition of the microbial community. Of the two treated areas, the decrease in soil respiration (laboratory measurements) was much more pronounced in the PB area. However, when the humus samples from both areas were adjusted to 60% water holding capacity, no differences in respiration capacity were observed. The drier humus, due to higher soil temperatures, of the PB area is a likely explanation for the low soil respiration. Lower soil respiration was not reflected in lower litter decomposition rates of the PB area, since there was a significantly higher needle litter mass loss during the first year in the PB area followed by a decline to the control level during the second year. Consistently higher mass losses were recorded in the FC area than in the FF area.

PIETIKÄINEN, J., et FRITZE, H. 1993. Microbial biomass and activity in the humus layer following burning: short-term effects of two different fires. Can. J. For. Res. **23** : 1275–1285.

Au cours d'une étude d'une durée de 3 ans, la quantité de C et de N dans la biomasse microbienne du sol, la longueur des hyphes fongiques, la respiration du sol ainsi que le pourcentage de perte de poids des couches d'humus du sol d'une forêt de conifères ont été notés suite à des traitements (cinq parcelles par traitement) comportant un brûlage dirigé (PB) ou un feu de forêt simulé (FF). L'humus non brûlé dans des parcelles adjacentes a servi de témoin (PC et FC respectivement). Le brûlage dirigé a été plus intense que le feu de forêt et cette situation s'est reflétée dans toutes les données récoltées. La quantité de C et de N dans la biomasse microbienne, la longueur des hyphes fongiques et la respiration du sol ne sont pas revenues au niveau témoin dans la zone soumise au traitement PB tandis que la quantité de N dans la biomasse microbienne est demeurée inchangée et que la longueur des hyphes fongiques est revenue au niveau témoin dans la zone soumise au traitement FF. Le ratio C/N moyen d'origine microbienne était d'environ 7 dans les deux zones traitées et reflétait le ratio C/N de la population microbienne du sol. Toute déviation de cette valeur moyenne, telle qu'observée lors des trois premiers échantillonnages, soit 3, 18 et 35 jours après le feu, suggérait qu'un changement était survenu dans la composition de la population microbienne. Des deux zones traitées, la diminution dans la respiration de sol, mesurée au laboratoire, était beaucoup plus forte dans la zone qui avait été soumise au traitement PB. Cependant, lorsque les échantillons d'humus en provenance des deux zones étaient ajustés à 60% de capacité de rétention d'eau, aucune différence dans leur capacité de respiration n'était observée. La faible respiration du sol observée dans la zone soumise au traitement PB est probablement due à un humus plus sec à cause de la température plus élevée du sol. La plus faible respiration du sol dans la zone soumise au traitement PB ne se traduisait pas par des taux de décomposition plus bas, étant donné que, dans la zone soumise à ce traitement, la perte de poids de la litière d'aiguilles était significativement plus élevée pendant la première année, suivie par une baisse jusqu'au niveau témoin pendant la deuxième année. Des pertes de poids plus élevées que dans la zone soumise au traitement FF ont toujours été observées dans la zone soumise au traitement FC.

[Traduit par la rédaction]

Introduction

In boreal forest ecosystems, podzolization is normally accompanied by the immobilization of essential nutrients, particularly carbon and nitrogen, within the soil humus layer. Podzolic soils are also characterized by their low bulk pH, which is mainly due to the formation of plant-derived humic and fulvic substances following litter decomposition and to cation uptake by plant roots, in combination with increased H^+ production in the rhizosphere (Tamm 1989). Both mineralization and humification of low molecular weight organic acids are inhibited by unfavourable conditions such as low temperature, high rainfall, or low soil nutrient status. In such situations, organic acids play an important role in soil acidification through deprotonating even at low soil pH (de Vries and Breeuwsma 1987). There natural processes, together with an increasing acidic deposition load, lead to soil acidification. In the past, succession to spruce-dominated forests in northern Scandinavia was normally interrupted by forest fires, which regularly occurred at intervals of approximately 80 years on average (Zackrisson 1977). However, because of fire prevention

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TABLE 1. Analysis of variance (repeated measures design)

		Ca		Mg		K		Na		C		N		N_{tot}	
Source[a]	df	SS	*F*	SS	*F*	SS	*F*	SS	*F*	SS	*F*	SS	*F*	SS	*F*
A	1	46.453	134.1*	22.276	156.1*	3.834 $\times 10^{-2}$	0.2	270.34	0.65	2.8674 $\times 10^{-5}$	0.00	40.703	160.9*	381.35	39.0*
A×B	8	2.7704		1.1413		1.4990		3308.1		2.6554		2.0236		78.222	
C	11	6.4241	14.3*	2.3991	8.82*	6.2546	8.55*	8278.6	10.2*	3.0245	3.44*	15.934	8.49*	53.306	3.12*
A×B×C	99	4.0336		2.4494		6.5816		7268.0		7.9163		16.897		153.52	

NOTE: See Fig. 7B caption for the definitions of the variable abbreviations.
[a] A, fire treatment; B, replicates in the error term; C, time after fire.
*$p < 0.05$.

policies, natural fires now rarely occur. Prescribed burning, as used in silvicultural practices, mimics natural forest fires because in both cases part of the humus is burned, causing the deposition of an ash layer on the soil surface. Burning results in the reduction in the thickness of humus layer and in an increase of its pH because of the release of basic cations (Viro 1974), which interrupts the natural process of soil acidification. Thus, prevention of natural forest fires and the decline in prescribed burning practices may have led to accelerated, natural soil acidification (Krug and Fink 1983). More recently, prescribed burning has been increasingly used as a tool to counteract soil acidification. As the microbial biomass mediates important soil biological processes, it is important to determine the impact of fire treatments on the soil microbial biomass and activity.

In this study we report on fire-induced changes in soil microbial biomass C and N, soil respiration, fungal hyphal lengths, and coniferous needle litter decomposition rate in two coniferous forest humus layers. Measurements were taken during the growing season in each of 3 consecutive years following two different fire treatments. The main objectives of the study were (*i*) to determine the effect of two different fire treatments on the soil microbial biomass and activity and (*ii*) to determine the duration of the effect.

Material and methods

Site description and soil sampling

Prescribed burning

The study was carried out at Evo (61°12′N, 25°7′E) in a 5-ha clear-cut area. The stand, dominated by Norway spruce (*Picea abies*), had been clear-cut in 1988 and burned on 25 May 1989. A total of 10 plots (on average 200 m^2 per plot) were established, of which 5 were located in the burned area (PB) and the rest served as controls in an adjacent *Myrtillus*-type spruce stand (PC). The soil was a Podzol developed on till material. Ten samples were collected (soil core diameter 7.2 cm) from the humus layer (F–H horizon for the controls, humus with ash layer for the treated plots) of each plot and were combined to give a composite sample as described by Fritze et al. (1992). During some samplings, more than 10 subsamples had to be taken from the burned plots to provide sufficient material for analyses. The plots were sampled six, four, and two times during the growing seasons of 1989 (once in May, twice in June, and once in July, August, and September), 1990 (June, July, August, and September), and 1991 (June and July), respectively. During each sampling, the temperature from the middle of the humus layer was recorded. To convert the measurements to a hectare basis, the thickness of the humus layer and the adjusted density, D_f (Macadam 1987), of the samples were determined once in July 1991 from 10 measurements per plot.

Simulated forest fire

The study was carried out in the Patvinsuo National Park (63°07′N, 30°40′E), where on 27 June 1989 a forest fire was simulated in a 1.1-ha forest area that was surrounded by a swamp. The forest, sparsely covered by silver birch (*Betula pendula*), was mainly dominated by Scots pine (*Pinus sylvestris*) and also Norway spruce in the northeast sector of the area. The main understory vegetation was *Vaccinium vitis-idaea*, *Vaccinium myrtillus*, and *Ledum palustre*. The soil was a Podzol on till. As in the Evo study area, 10 plots were established; 5 in the treated area (FF) and 5 in an adjacent *Vaccinium–Myrtillus* type pine stand, which served as the control area (FC). The soil samples were collected as already described; at this location 10 core samples from each plot was sufficient for analysis. The plots were sampled on four (June, July, August, and September), four (June, July, August, and September), and two (June and July) occasions during the growing seasons of 1989, 1990, and 1991, respectively. The thickness and adjusted density of the humus layer were measured in July 1991 as described above.

Soil analyses

The samples were sieved (mesh size 4 mm), and visible plant material was removed before storage at 4°C. Moisture content was determined from subsamples that were dried at 105°C for 12 h. For the determination of the organic content a 1-g portion of dried humus was ignited at 550°C for a minimum of 3 h, and the loss on ignition was determined. Sample density was determined by weighing 5-cm^3 aliquots of dried homogenized humus, and the values were used in the microbial biomass determination. The cations Ca, Mg, K, Na were extracted from replicate 5-g dry weight equalling air-dried samples with 100 mL 1 M ammonium acetate (pH 4.65) and quantified by atomic absorption spectrophotometry. Total N was analyzed by the Kjeldahl method. The extractable amounts (organic and mineral) of C and N were obtained from unfumigated samples in the microbial biomass determination (see below). Soil pH was determined in soil–water suspensions (1:10, w/v).

All soil biological determinations were initiated within 5 days of sample collection. The production of CO_2 was measured by gas chromatography on triplicate 2.5-g samples of field-moist humus that had been incubated at 14°C for 48 h in a closed 120-mL glass bottle, as described by Fritze et al. (1989). On two occasions the samples were adjusted, after the CO_2 determination, to a water holding capacity (WHC) of 60%, and after 48 h the soil respiration measurement was repeated.

The 0.5 M K_2SO_4 extractable C and N, from the chloroform-fumigated samples and their unfumigated controls, was converted to microbial C and N using the sample density and the equations described by Martikainen and Palojärvi (1990). Three replicates were

of the variables from the prescribed burning area

pH		T		BMC		BMN		CO_2		qCO_2		Hyphae		
SS	*F*	SS	*F*	SS	*F*	SS	*F*	SS	*F*	SS	*F*	df	SS	*F*
93.316	202.6*	1360.1	1594*	1.7307 $\times 10^8$	331.2*	6.0037 $\times 10^6$	447.1*	5077.0	26.6*	3.8186	35.02*	1	1.5375 $\times 10^8$	19.8*
3.6838		6.8279		4.1808 $\times 10^6$		1.0743 $\times 10^5$		1527.8		8.7243 $\times 10^{-1}$		8	6.2094 $\times 10^7$	
9.0851	7.05*	1708.1	67.4*	1.8865 $\times 10^7$	2.87*	1.8321 $\times 10^5$	1.17	2.0557 $\times 10^4$	7.79*	39.352	7.67*	8	1.0454 $\times 10^8$	7.77*
11.594		223.54		5.9243 $\times 10^7$		1.4079 $\times 10^6$		2.3762 $\times 10^4$		44.304		71	1.1941 $\times 10^8$	

made for each analysis. Organic C was determined on an Uras instrument after filtering each extract through a millipore membrane (pore size 0.2 μm), as described by Salonen (1979). Total N of the extract was determined by the Kjeldahl method.

Fungal hyphal length estimations were based on the method described by Sundman and Sivelä (1978) following sample preparation as described by Fritze et al. (1989). Three replicates were prepared for each analysis. A mean hyphal diameter of 2.5 μm was calculated from 60 independent measurements. When the density of the hyphae was taken as 1.1 $g \cdot cm^{-3}$, the dry weight as 15% of the fresh weight, and the content of C as 45% of the dry weight (see Martikainen and Palojärvi 1990), a crude estimate of the fungal biomass C within the total microbial biomass C could be calculated.

Two-year-old green Scots pine and Norway spruce needles representing the growth in 1986 were collected in March 1988 from a background area, as described by Fritze (1988). Sixty litter bags (70 × 70 mm), made of woven polyamide material with a mesh size of 1 × 0.8 mm, each containing 1 g of dried (60°C, overnight) needle material, were buried in the middle of the humus layer of each plot. Twenty bags were collected per year from each plot in 1990 and 1991, and their weight loss was calculated after drying at 60°C overnight. Norway spruce and Scots pine needles were used in the prescribed and the simulated fire experiment, respectively.

Statistical analysis

The data ($n = 220$, except hyphal length, where $n = 160$) were tested for homoscedasticity, and the following variables needed a logarithmic (ln) transformation to improve their normality: Ca, Mg, K, C_{extr}, N_{extr}, and the ratio of soil respiration rate to microbial biomass C (qCO_2) in the prescribed burning set and K in the simulated forest fire set, respectively. Analysis of variance (ANOVA) was performed as a repeated measures design to estimate the impact of fire treatment and time of sampling on the measured variables. The mass loss of the needle litter was subjected to one-way ANOVA. The difference between the forest fire and its control area with respect to the length of the fungal hyphae during the last two samplings was tested by ANOVA. The Statistix 3.1 program (NH Analytical Software) was used. The means ($n = 44$) of the variables, except fungal hyphal length and mass loss of needle litter, from each of the four areas from every sampling were subjected to a principal component analysis. This reveals the total difference of the fire-treated plots from its controls and the reaction of the included variables. The variables were standardized to unit variance. The Sirius programme (Kvalheim and Karstand 1987) was used.

Results

As the humus layer is most affected by fire the results are presented on the basis of mass of organic matter (measured as loss on ignition). An appendix presents results calculated per gram soil dry weight and per hectare. The statistical analysis of the measured variables are presented in Tables 1 and 2.

The mean (SD) thickness of the humus layers in the PB and PC areas were (in cm) 2.7 (0.8) and 6.4 (0.4), respectively, and corresponding values in the FF and FC areas were 5.5 (0.6) and 8.1 (1.2), respectively. This revealed a 58% reduction in thickness of the humus layer after prescribed burning compared with only a 31% reduction following the simulated forest fire. The two fire treatments released different amounts of extractable cations into the humus layer when compared with the controls (Fig. 1). As a consequence of the fire, Ca levels increased 4- to 6-fold and Mg levels 3- to 4-fold in the PB area as opposed to only 2-fold increases of both elements in the FF area. An equivalent amount of extractable K was liberated after the two fire treatments in both cases. Losses of K from the humus layer through leaching were detected immediately after burning, and K concentration reached control levels within the first year. No fire-related differences in amounts of extractable Na could be detected.

The amount of extractable C (organic and mineral) in the humus layer of both fire treatments was higher immediately after burning (Figs. 2A and 2B), but rapidly decreased to control levels. In the PB area, increased levels of extractable N (organic and mineral) were initially recorded, which then declined to control values after 1.5 years. In contrast, elevated levels in the FF area were recorded immediately after burning but thereafter fell below the control values (Figs. 2C and 2D). In both fire treatments the total N concentration, when expressed per unit of organic matter, was greater than in their respective controls (Figs. 2E and 2F), which indicates that the burned soils have a lower C/N ratio than similar unburned soils.

A fire-induced shift of approximately 2 pH units was recorded in the humus layer of the PB area (from 4 to 6), and only 0.4 units in the FF area (from 3.8 to 4.2), when compared with their respective controls (Fig. 3). The pH shift persisted over the three growing seasons, although in the PB area the pH had declined to 5.2 by the end of the third year. The soil temperature, measured every sampling, had respective mean (SD) values (in °C) of 11.3 (3.2) and 18.1 (4.8) in the PC and PB areas compared with 12.0 (3.8) and 12.8 (3.5) in the FC and FF areas.

As a result of fire, decreased microbial biomass C and N were recorded in the humus layer (Figs. 4A–4D). The

TABLE 2. Analysis of variance (repeated-measures design)

		Ca		Mg		K		Na		C		N		N_{tot}	
Source[a]	df	SS	*F*	SS	*F*	SS	*F*	SS	*F*	SS	*F*	SS	*F*	SS	*F*
A	1	4.3311 $\times 10^7$	74.6*	1.3549 $\times 10^6$	32.5*	3.5902 $\times 10^{-1}$	6.81*	867.74	3.25	2.2404 $\times 10^6$	13.5*	6806.8	3.52	30.003	6.50*
A×B	8	4.6442 $\times 10^6$		3.3376 $\times 10^5$		4.2164 $\times 10^{-1}$		2138.5		1.3290 $\times 10^6$		1.5472 $\times 10^4$		36.927	
C	9	3.4009 $\times 10^7$	31.6*	9.6078 $\times 10^4$	2.24*	3.2055	8.43*	4291.0	3.51*	4.2989 $\times 10^6$	4.94*	7.8203 $\times 10^4$	4.90*	12.246	7.91*
A×B×C	81	9.6789 $\times 10^6$		3.8637 $\times 10^5$		3.4205		1.1017 $\times 10^4$		7.8390 $\times 10^6$		1.4375 $\times 10^5$		13.927	

NOTE: See Fig. 7B caption for the definitions of the variable abbreviations.
[a] A, fire treatment; B, replicates in the error term; C, time after fire.
*$p < 0.05$.

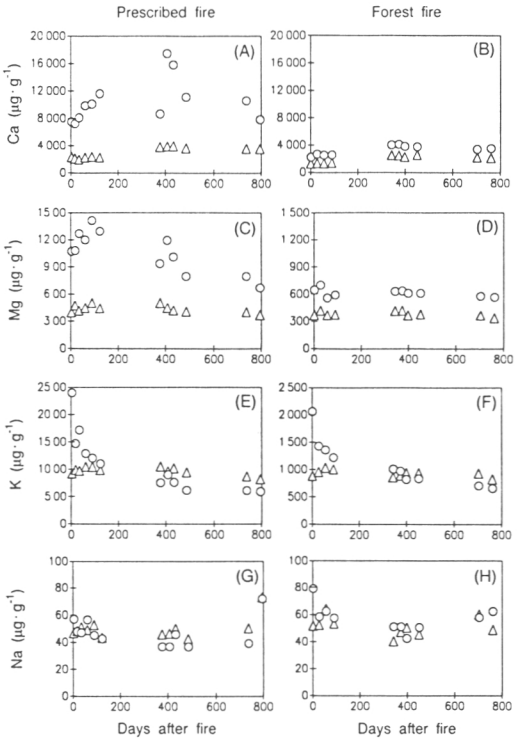

FIG. 1. Amounts of extractable Ca (A and B), Mg (C and D), K (E and F), and Na (G and H) in the humus layer of the prescribed burning and forest fire trials (organic matter was measured as loss on ignition). Values are means ($n = 5$) for the burned (○) and control (△) plots.

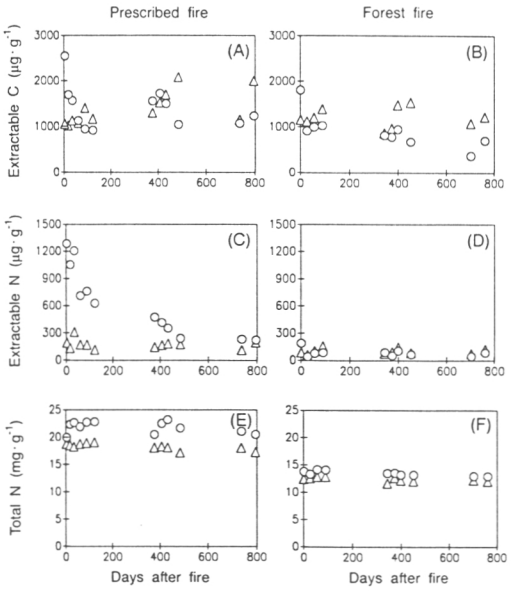

FIG. 2. Amounts of extractable C (A and B) and N (C and D), and total N (E and F) in the humus layer of the prescribed burning and forest fire trials (organic matter was measured as loss on ignition). Symbols are as in Fig. 1.

respective 3-year mean (SD) microbial biomass C totals for the PC and PB areas were (in $\mu g \cdot g^{-1}$) 5880 (930) and 3480 (728) and corresponding totals for the microbial biomass N were 935 (122) and 488 (117). No increase in microbial biomass C and N towards control levels was recorded in the PB area over the study period. The mean microbial biomass C of the FC area was 6177 (839) and of the FF area 4930 (589), and the corresponding biomass N values were 740 (113) and 700 (103), respectively. Microbial N did not differ significantly between the two areas.

As a result of the fire treatments the length of the fungal hyphae decreased in the humus layer (Figs. 4G–4H). The 3-year mean (SD) fungal hyphal lengths (in $m \cdot g^{-1}$) were 6141 (2188), 3525 (1325), 7423 (1575), and 5483 (1452) for the PC, PB, FC, and FF areas, respectively. The fungal hyphal length in the FF area reached control levels by the end of the second year. Hyphal lengths from the last two samplings of the FF area were not significantly different from those of the control area ($F = 0.41$, $p < 0.53$ and $F = 1.16$, $p < 0.31$, respectively). Around 40% of the microbial biomass C was present in the fungal biomass irrespective of the treatment.

of the variables from the simulated forest fire area

pH		T		BMC		BMN		CO_2		qCO_2		Hyphae		
SS	*F*	SS	*F*	SS	*F*	SS	*F*	SS	*F*	SS	*F*	df	SS	*F*
5.2556	119.9*	17.206	22.6*	3.8589 $\times 10^7$	17.7*	3.3043 $\times 10^4$	0.76	1366.3	7.50*	3.0700 $\times 10^{-7}$	0.13	1	6.5892 $\times 10^7$	13.1*
3.5066 $\times 10^{-1}$		6.0787		1.7449 $\times 10^7$		3.4559 $\times 10^5$		1457.4		1.8740 $\times 10^{-5}$		8	4.0297 $\times 10^7$	
3.3519 $\times 10^{-1}$	2.91*	1244.1	120.7*	9.2854 $\times 10^6$	3.37*	1.8477 $\times 10^5$	2.69*	3345.5	6.41*	1.3256 $\times 10^{-4}$	9.47*	6	1.6087 $\times 10^7$	1.45
1.0363		92.791		2.4780 $\times 10^7$		6.1787 $\times 10^5$		4700.8		1.2595 $\times 10^{-4}$		54	9.9737 $\times 10^7$	

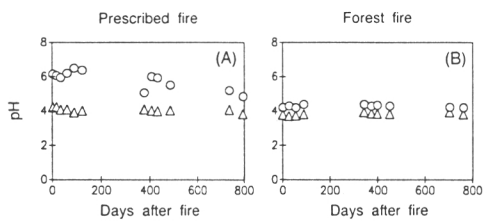

FIG. 3. The pH of the humus layer after burning (○) and their controls (△) in the prescribed burning (A) and forest fire (B) trials.

In the PB area the C/N ratio of the microbial biomass (biomass C / biomass N) in the burned organic matter remained above that of the control area during the first month (samplings from 3, 18, and 35 days) after burning, declining from 12 towards control levels. This could not be observed in the FF area. The 3-year mean microbial C/N ratios of the PC, PB, FC, and FF areas were 6.4 (1.3), 7.1 (1.4), 8.5 (1.4), and 7.1 (1.1), respectively.

By dividing the value of the microbial biomass N with the corresponding soil total N value, estimates of the mean (SD) percent total soil N immobilized in the microbial biomass were calculated to be 5.1 (0.7), 2.2 (0.5), 5.9 (0.8), and 5.3 (0.9) for the PC, PB, FC, and FF areas, respectively.

The respiration of field-moist soil rapidly fell below the control levels in both fire treatments (Figs. 4E and 4F) and showed no recovery with time. Only the first samplings after the fire treatments showed a higher respiration rate than in the controls. The 3-year means (SD) of the soil respiration rate were (in $\mu L\ CO_2 \cdot g^{-1} \cdot h^{-1}$) 40.2 (10.1), 27.2 (25.9), 34.4 (8.72), and 27.0 (10.8) in the PC, PB, FC, and FF areas, respectively. From these values the average decrease in soil respiration in the PB and FF areas, when compared with their control areas, were 32.3 and 21.5%, respectively. When the soil was adjusted to 60% WHC, the difference between PB and PC areas was reduced to the same level as between the FF and FC areas (Fig. 5).

The ratio of the soil respiration rate to microbial biomass C (qCO_2) showed a linear relationship with time in the control areas but a logarithmic relationship in the fire-treated areas (Fig. 6), with decreased qCO^2 during the first growing season.

In the first year of soil incubation, needle litter mass loss was significantly greater in the PB area than in the adjacent

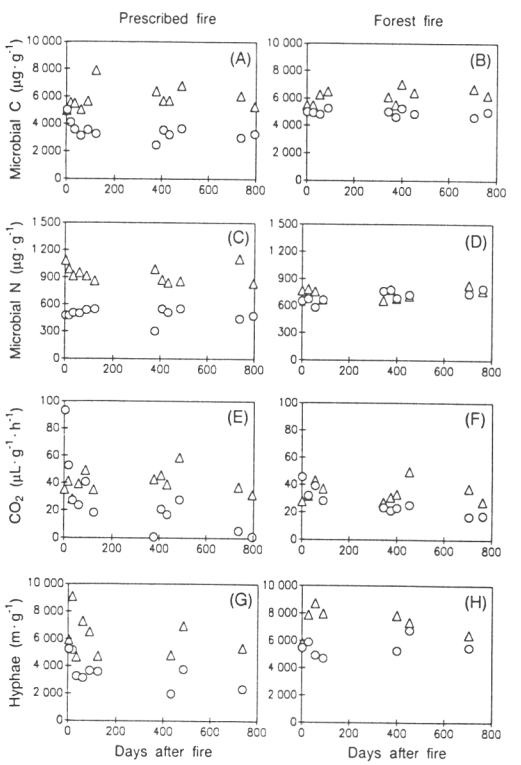

FIG. 4. Microbial biomass C (A and B) and N (C and D), soil respiration (E and F), and the length of fungal hyphae (G and H) in the humus layer of the prescribed burning and forest fire trials (organic matter was measured as loss on ignition). Symbols are as in Fig. 1.

control area (PC), although this difference disappeared during the second year of soil incubation (Table 3). In contrast, the needle litter mass loss of the FF area exhibited significantly lower rates than its control area (FC) during both years of soil incubation.

The mean values of each variable, except the fungal hyphal length and needle litter mass loss data, from all sampling

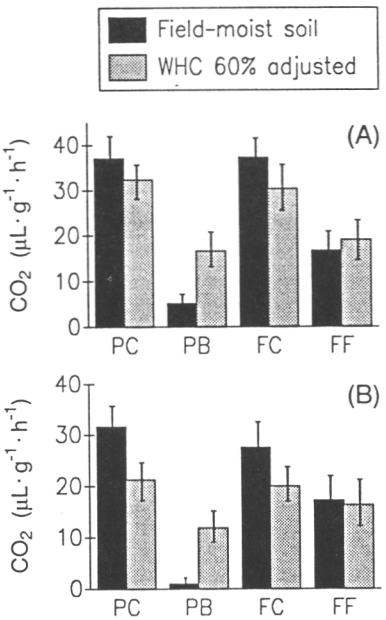

FIG. 5. Comparisons between respiration levels of field-moist humus and humus adjusted to 60% water holding capacity (WHC) during two samplings (A and B) from the prescribed control (PC), prescribed burning (PB), forest fire control (FC), and forest fire (FF) areas (organic matter was measured as loss on ignition). Standard deviation is indicated.

occasions were subjected to PC analysis (Figs. 7A and 7B). Nearly 70% of the total variance in the data set was explained in the first two axes. The first component accounted for 43.7% of variance in the total data, whereas the second component increased the variance by 25.6%. The first component represented the impact of the fire on the soil data, since variables showing fire-induced changes received high loadings (i.e., pH, Mg, Ca, microbial biomass C, N_{tot}). The second component represented a combination of fire and the time after the fire effects, since variables influenced by the fire and showing time-dependent changes received high loadings on this component (i.e., CO_2 evolution, extractable K, qCO_2). High loadings for extractable N were seen in both components. The first component separates the PB plots on the left from all the other plots (PC, FC, FF), which were closely clustered on the right of the origin (Fig. 7A). This indicates that fire treatment had the greatest influence on chemical and microbiological variables in the PB plots, where also the fire intensity was the greatest. The FF plots are on the left of the cluster, indicating that the fire treatment had a small influence on the characteristics measured. The second component separates the PB plots into a time-dependent series with PB3 (3 days after burning) and PB796 (796 days after burning) at the opposite ends of the figure. The FF plots are also similarly separated by the second component, but to a smaller extent than the PB plots. Figure 7B shows the reaction of the measured variables on the two components, condensing the information of the whole data into one figure. Variables with negative scores on the first axis are elevated as a result of the fire treatment, whereas variables with high negative scores on the second axis show a rapid decline to or below the control levels. The length of the vector between the origin and the variables positively correlates with the response of the variable to the treatment.

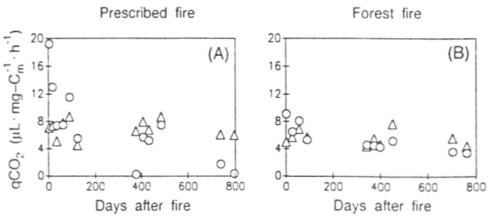

FIG. 6. Development of the metabolic quotient (qCO_2) after prescribed burning (A) and forest fire treatments (B). Symbols are as in Fig. 1.

TABLE 3. Study area mean percent mass loss (n = 100 year^{-1}) of coniferous needle litter buried in the humus layer of the five plots

Years since fire	PB	PC	FF	FC
1	35.3* (3.9)[a]	31.7 (2.9)	31.2* (6.4)[b]	38.6 (3.6)
2	50.7 (11.5)[c]	50.3 (9.1)	47.2* (6.0)[d]	52.5 (6.7)

NOTE: Standard deviation is in parentheses. PB, prescribed fire, burned area; PC, prescribed fire, control area; FF, forest fire, burned area; FC, forest fire, control area.
[a] $F_{[199]} = 53.9$.
[b] $F_{[199]} = 99.6$.
[c] $F_{[199]} = 0.09$.
[d] $F_{[199]} = 34.3$.
*Means are significantly different ($p < 0.05$, ANOVA) from their controls.

Discussion

The intensity of a fire, measured on the basis of energy released per unit time, is known to play a major role in determining the effects of any fire treatment on soil (Chandler et al. 1983). In addition to a reduction in thickness, combustion of the humus layer releases extractable Ca and Mg, originating from the humus and the aboveground plant vegetation. Compared with the FF area the PB area had a more pronounced reduction in the thickness of the humus layer, which correlated with the higher concentrations of extractable Ca and Mg originating from a higher amount of burned fuel. This suggested that the intensity of the prescribed burning was higher than that of the simulated forest fire, although fire intensity was not directly measured in this study. This is also clearly suggested in the PC analysis of the whole data set (Fig. 7). In addition to the elevated Ca and Mg levels, the soil bulk pH of the PB area was also higher than that of the FF area (Fig. 3). Immediately after both fires, there were higher extractable K levels which then declined considerably during the first growing season. Extractable Na levels showed no clear differences between fire-treated and control areas, indicating that the relationship between organic matter and Na had not changed. It was not the aim of this study to discuss the observed differences in the respective cations and pH in the investigated areas, since they were analyzed only to provide fire correlative data. Instead we focus on the microbial biomass and its activity in these two differently burned soils.

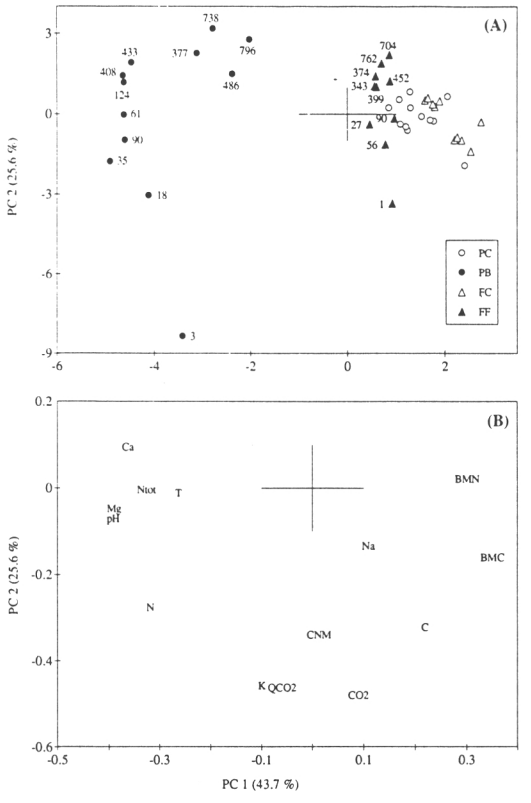

FIG. 7. Biplot based on principal component analysis showing the separation of the plots (A) and the separation of the analyzed variables (B) by the two axes. The prescribed burning (PB) and forest fire (FF) plots are indexed by a number refering to the days after fire (A). Ca, Mg, K, Na, N, and C are the extractable amounts of these elements as presented in Figs. 1 and 2. *T*, pH, and N_{tot} represent soil temperature, pH, and total soil N, respectively. BMN, BMC, CNM, CO2, and QCO2 represent microbial biomass N, microbial biomass C, C/N ratio of the microbial biomass, soil respiration, and the metabolic quotient, respectively (B).

After the fire treatments microbial biomass C and N, and the fungal hyphal lengths, declined below the control levels, and recovery to control levels was only detected for biomass N and the length of fungal hyphae in the FF area. The mean microbial C/N ratio was around 7 in all the areas, which is within the range that Martikainen and Palojärvi (1990) report as the mean ratio of $CHCl_3$-induced (= microbial-derived) release of extractable C to extractable N for coniferous, deciduous, and arable soils. However, the first three samplings (3, 18, and 35 days after fire treatment at the PB area) showed much higher microbial C/N ratios of 12, 9, and 8.5, respectively, which thereafter returned to the mean level of 7. This was only observed in the more heavily burned PB area. Although Tate et al. (1988) have indicated that there are differences in the C/N ratio of different microbes, the value of 7 reflects the C/N ratio of the microbial community. Thus, a deviation from the observed mean value may indicate a change in the composition of the microbial community.

Prescribed burning has been shown to induce a number of changes in soil microbial populations of the humus layer. Immediate effects of fire are seen in the heat-induced reduction of soil bacterial numbers (Meiklejohn 1955; Wright and Bollen 1961; Ahlgren and Ahlgren 1965; Jorgensen and Hodges 1970; Sharma 1981; Theodorou and Bowen 1982; Deka and Mishra 1983). Recovery of bacterial counts to or above control levels seems to occur within 1 month but can also take between 12 to 14 months. The speed of recovery is greatly dependent on soil moisture, as the initial population decline usually continues until the first rainfall (Ahlgren 1974). According to Dunn et al. (1985), fungi showed greater heat sensitivity than heterotrophic bacteria when soil was heated to various temperatures. All studies, except Jorgensen and Hodges (1970), report a decrease of fungal propagules in the soil following fire (Meiklejohn 1955; Wright and Bollen 1961; Ahlgren and Ahlgren 1965; Jalaluddin 1969). Additionally, rapid recovery in the number of fungal propagules after the fire treatment occurs, but changes in the species composition of fungal population, when compared with control soils, are to be seen over a longer period after the fire treatment (Widden and Parkinson 1975; Tiwary and Rai 1977; Bisset and Parkinson 1980). We propose that our observed difference in microbial C/N ratio during the first month after the fire treatment could reflect a rapidly changing microbial species composition. This would also suggest that the species composition was not so dramatically affected in the less intensively burned FF area.

The soil microbial biomass acts as a sink for nutrients. In most arable soils the microbial biomass C represents only about 1–3% of the soil organic C (Anderson and Domsch 1989), and the values for coniferous and deciduous forest soils fall into the same range (Vance et al. 1987; Martikainen and Palojärvi 1990). Our data did not permit an estimation of the amount of microbial soil C immobilization, as the total soil C content was not determined, but the proportion of microbial biomass N immobilized was 5.1, 2.2, 5.9, and 5.3% of the soil total N in the PC, PB, FC, and FF areas, respectively. The values of the three homogenous areas closely correspond with the value 5.9 reported for coniferous soils, but the value of the PB area comes close to the value of 2.5 reported for arable soils (Martikainen and Palojärvi 1990). Chander and Brookes (1991) use changes in the ratio of biomass C to soil organic C as an index for changes in conditions of soils exposed to chemical pollution, heavy metals, or other disturbances. The ratio, derived in our case from the ratio of microbial N to soil total N shows that the mean soil condition deviations from the control levels were 12.2% for the forest fire and 56.3% for the prescribed burning, which corresponds to the results from the whole data set when analyzed by principal component analyses (Fig. 7).

The activity of the soil microbial biomass can be estimated from soil respiration measurements and decomposition rates of organic material buried in the humus layer. To date the effect of fire on soil metabolism has been the subject of relatively few studies. Ahlgren and Ahlgren (1965) measured laboratory respiration rates of soils sampled at intervals over a 3-year period following fire in a jack pine (*Pinus banksiana*) forest. They found initial decreases in respiration rates after fire, but later, after the first rainfall, a rapid recovery occurred

to levels above those of the controls. Consistently lower respiration rates in ponderosa pine soil, compared with preburned values, were reported during the first year after a fire (White 1986). Bisset and Parkinson (1980) reported that 6 years after the fire treatment, respiration in subalpine coniferous forest sites, as measured in the laboratory, remained below control levels. In situ measurements of soil respiration revealed no clear fire-induced differences in jack pine ecosystems (Weber 1985), but in a study comparing burned, clear-cut, and control immature aspen ecosystems the clear-cut and the burned sites showed lowered soil respiration rates over the first two growing seasons after the fire (Weber 1990). The soil respiration, as assessed on field-moist humus samples, decreased below control levels during the first month after fire at both treatment areas and remained so over the 3 years. This reduction in soil respiration was much more pronounced in the PB area than in the FF area, although when the soils were adjusted to 60% WHC, the respiration capacity of the PB area was elevated to that of the FF area. The higher soil temperatures and consequently the drier humus of the PB area partly explain the low soil respiration. Elevated soil respiration rates can be expected in the PB area after rain but not in the FF area, where the adjustment to 60% WHC had no effect on the soil respiration rate.

Although reduced soil respiration would suggest a lowered decomposition rate of buried organic material in the PB area, this was not consistently the case in our study. During the first year a significantly higher mass loss of needle litter material occurred in the PB area, which in the second year declined to control levels. Higher decomposition rate of cellulose, in 3-month soil incubations, was reported in burned sites as compared with unburned controls (Bisset and Parkinson 1985). Weber (1987) started assessing the weight loss of overstory litter in jack pine soils several years (minimum 15 years) after they had received the fire treatment and found no differences between the sites. In the first phase of decomposition the mass loss rate of coniferous needle litter is mainly limited by the low concentration of litter N (Berg 1986). Decomposers must therefore import N from the immediate surroundings. We suggest that the observed higher mass loss rate in the PB area during the first year is due to favourable conditions of higher soil temperature, higher availability of soil nitrogen, and to a microflora characterized by a high turnover of readily decomposable carbon sources (see below). The second phase of coniferous litter decomposition, from the second year onwards, is dependent on the litter lignin concentration. Two factors that influence the decomposition rate of lignin are (*i*) high N concentration, which retards decomposition and (*ii*) high concentration of cellulose, which increases the turnover rate (Berg 1986). In the second year the PB area still had higher soil N concentrations and less extractable carbon than the PC area. This could explain the observed decrease of the needle litter mass loss to nearer control levels in the second year of soil incubation. We have no good explanation as to why the needle litter mass loss in the FC area was consistently higher than in the FF area. It is possible that the C/N ratio of the soil in the FF area did not decrease enough to permit a faster mass loss rate during the first year.

The ratio of microbial respiration to biomass C (qCO_2) is related to environmental stress in polluted soils (Visser and Parkinson 1989; Insam 1990; Fritze 1991; Fritze et al. 1992) and to soil development, where decreased values are linked with ecological succession (Insam and Domsch 1988; Insam and Haselwandter 1989). More developed soils evolve less respiratory CO_2 per unit maintained microbial C than young soils. The high observed qCO_2 values, especially in the PB area, suggests that there is a state of rapid succession in the first year after fire, which is reflected in the higher qCO_2 seen in the burned areas. The difference in the ratio would probably have been more pronounced and the time lag of the decline would have taken longer between the PB and PC areas if the quotient had been derived from soils adjusted to 60% WHC. In a trial where soil samples were taken in a time series between 0 and 45 years after prescribed burning and adjusted to 60% WHC, it took over 2 years for the qCO_2 to decline towards control levels (Fritze et al. 1993). In our opinion the observed high qCO_2 during the first year after the fire in the PB area also suggests the existence of a microbial population specialized on readily decomposable carbon sources for maintenance of the microbial biomass. The time-dependent decline of the qCO_2, compared with the more straight line relationship of the respective controls in these data, highlights the use of readily decomposable carbon sources during the first year after fire. Later when the qCO_2 of the fire-treated areas show no change with time, as seen in the controls, it is likely that the carbon sources utilized by the biomass are similar to those in the control soils.

In the burned soils the average time span of fire-induced effects on the measured biological variables was over 3 years. An estimate of the duration could be obtained by plotting the first principal component, which separated the PB plots from the others (Fig. 7), against time. Then the PB plots showed a decline towards the level of the FF, FC, and PC plots that would be reached within 5 years of the fire treatment.

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Appendix

TABLE A1. Means expressed on a soil dry weight basis

Days after fire	Ca ($\mu g \cdot g^{-1}$)	Mg ($\mu g \cdot g^{-1}$)	K ($\mu g \cdot g^{-1}$)	Na ($\mu g \cdot g^{-1}$)	Extr. N ($\mu g \cdot g^{-1}$)	Extr. C ($\mu g \cdot g^{-1}$)	Total N ($mg \cdot g^{-1}$)	CO_2 ($\mu L \cdot g^{-1} \cdot h^{-1}$)	N_{mic} ($\mu g \cdot g^{-1}$)	C_{mic} ($\mu g \cdot g^{-1}$)	Hyphae ($m \cdot g^{-1}$)
Prescribed fire, controls											
3	1865	318	740	36.8	156	850	15.1	28.4	869	3980	4620
18	1749	392	812	40.1	108	827	15.3	33.8	814	4555	7538
35	1651	349	814	42.3	263	951	15.3	23.7	761	4612	3840
61	1795	358	837	39.0	136	856	15.0	31.1	755	4015	5760
90	1726	369	768	39.1	126	1036	13.8	36.2	666	4130	4722
124	1776	346	774	33.7	88.4	920	14.9	27.5	685	6256	3532
377	2846	384	789	34.6	108	981	13.6	32.0	745	4848	nd
408	3126	362	772	37.0	134	1224	14.7	36.5	698	4551	nd
433	3088	328	789	38.2	144	1326	14.1	30.1	654	4447	3688
486	3066	340	793	35.0	147	1764	14.3	48.8	717	5709	5637
738	2854	324	695	40.5	89.5	913	14.4	29.7	880	4839	4318
796	2834	298	658	59.1	159	1626	13.9	25.5	677	4264	nd

TABLE A1 (*concluded*)

Days after fire	Ca ($\mu g \cdot g^{-1}$)	Mg ($\mu g \cdot g^{-1}$)	K ($\mu g \cdot g^{-1}$)	Na ($\mu g \cdot g^{-1}$)	Extr. N ($\mu g \cdot g^{-1}$)	Extr. C ($\mu g \cdot g^{-1}$)	Total N ($mg \cdot g^{-1}$)	CO_2 ($\mu L \cdot g^{-1} \cdot h^{-1}$)	N_{mic} ($\mu g \cdot g^{-1}$)	C_{mic} ($\mu g \cdot g^{-1}$)	Hyphae ($m \cdot g^{-1}$)
Prescribed fire, burned											
3	5187	738	1653	39.2	885	1755	13.7	64.1	329	3449	3558
18	4206	638	859	27.9	606	998	13.0	30.6	270	2370	2991
35	5020	789	1077	29.5	756	982	14.4	16.4	311	2229	2058
61	6106	750	800	35.0	433	684	13.7	14.6	315	1974	1991
90	5877	827	715	26.5	446	550	13.3	23.7	310	2067	2148
124	6335	709	598	23.4	344	496	12.4	10.0	297	1778	1923
377	6222	676	547	26.6	341	1116	14.8	0.462	218	1777	nd
408	9018	636	476	19.6	224	901	12.1	10.7	296	1937	nd
433	8156	512	389	23.2	179	769	11.8	8.64	260	1637	989
486	6886	492	384	22.8	153	648	13.4	17.1	340	2260	2321
738	5264	396	302	19.4	120	531	10.6	2.51	222	1500	1104
796	4688	402	357	43.4	139	742	12.3	0.526	283	1980	nd
Forest fire, controls											
1	1115	322	770	45.2	75.4	1012	10.9	24.1	669	4821	5805
27	1174	367	836	45.9	52.6	986	11.0	27.6	689	4820	7873
56	1153	322	905	56.4	89.0	1054	11.2	37.8	666	5497	8714
90	1199	328	886	47.1	135.0	1207	11.3	32.5	589	5710	7962
343	2122	354	736	34.8	63.8	743	9.98	23.2	567	5256	nd
374	2126	362	769	41.0	79.1	829	10.9	26.5	673	4805	nd
399	1984	320	825	44.6	127.0	1303	10.7	29.1	602	6214	7839
452	2272	334	838	40.6	74.0	1371	10.7	44.2	629	5720	7345
704	1964	326	823	53.2	65.1	958	10.9	33.1	739	5967	6423
762	1820	292	712	42.6	107.0	1060	10.3	23.7	666	5382	nd
Forest fire, burned											
1	1958	578	1844	70.5	166.0	1607	12.3	40.6	582	4462	5458
27	2309	604	1232	50.1	33.6	787	11.4	27.5	580	4258	5881
56	2244	497	1208	55.2	68.4	885	12.5	34.7	515	4279	4908
90	2275	532	1095	50.9	75.1	918	12.6	25.2	595	4730	4679
343	3418	542	862	43.2	73.1	698	11.4	19.8	648	4279	nd
374	3582	562	862	45.0	44.7	688	11.9	18.6	686	4082	nd
399	3318	536	713	36.6	93.0	823	11.4	20.1	595	4577	5250
452	3264	536	730	44.0	55.6	589	11.4	21.9	637	4277	6733
704	3008	512	623	51.4	38.4	327	11.4	14.7	654	4092	5469
762	2964	478	554	52.2	70.2	586	10.8	14.4	668	4216	nd

NOTE: Extr., extractable; nd, not determined.

TABLE A2. Means expressed on an area basis

Days after fire	Ca ($kg \cdot ha^{-1}$)	Mg ($kg \cdot ha^{-1}$)	K ($kg \cdot ha^{-1}$)	Na ($kg \cdot ha^{-1}$)	Extr. N ($kg \cdot ha^{-1}$)	Extr. C ($kg \cdot ha^{-1}$)	Total N ($kg \cdot ha^{-1}$)	CO_2 ($L \cdot ha^{-1} \cdot h^{-1}$)	N_{mic} ($kg \cdot ha^{-1}$)	C_{mic} ($kg \cdot ha^{-1}$)	Hyphae ($10^9\ m \cdot ha^{-1}$)
Prescribed fire, controls											
3	99.5	16.7	39.3	1.95	8.16	45.6	799	1513	46.5	212	247
18	92.3	20.5	43.0	2.10	5.76	44.3	804	1788	43.1	241	393
35	87.4	18.4	43.0	2.25	13.6	50.2	808	1247	40.3	244	205
61	95.0	18.8	44.6	2.08	7.49	46.2	793	1681	40.3	213	308
90	93.1	19.6	40.7	2.03	6.62	55.0	735	1928	35.4	220	256
124	94.3	18.2	40.9	1.79	4.81	48.9	790	1458	35.8	330	193
377	150	20.0	41.6	1.83	5.69	51.9	719	1694	39.4	255	nd
408	166	19.0	41.3	1.97	7.13	66.2	779	1960	37.3	243	nd
433	162	17.2	42.1	2.04	7.69	70.7	745	1611	34.6	235	195
486	160	17.9	41.6	1.86	7.74	92.4	754	2587	37.7	299	299
738	150	16.9	36.7	2.12	4.75	48.4	760	1570	46.7	256	228
796	151	15.8	34.8	3.09	8.41	85.9	736	1356	35.8	225	nd

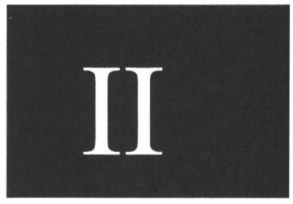
TABLE A2 (*concluded*)

Days after fire	Ca ($kg \cdot ha^{-1}$)	Mg ($kg \cdot ha^{-1}$)	K ($kg \cdot ha^{-1}$)	Na ($kg \cdot ha^{-1}$)	Extr. N ($kg \cdot ha^{-1}$)	Extr. C ($kg \cdot ha^{-1}$)	Total N ($kg \cdot ha^{-1}$)	CO_2 ($L \cdot ha^{-1} \cdot h^{-1}$)	N_{mic} ($kg \cdot ha^{-1}$)	C_{mic} ($kg \cdot ha^{-1}$)	Hyphae (10^9 $m \cdot ha^{-1}$)
Prescribed fire, burned											
3	274	38.7	86.3	2.04	46.8	92.0	723	3358	16.7	181	183
18	217	33.2	45.3	1.47	31.5	52.5	687	1589	14.1	124	157
35	261	41.2	56.7	1.56	39.8	51.4	769	841	16.0	116	109
61	322	39.8	42.5	1.81	22.9	35.9	724	770	16.5	104	105
90	311	43.7	37.8	1.41	23.4	29.0	707	1250	16.0	108	114
124	334	37.3	31.7	1.24	17.8	25.9	658	520	15.8	92.8	102
377	324	35.5	28.9	1.41	17.9	58.6	787	24.7	11.5	94.1	nd
408	470	33.4	25.1	1.03	11.6	46.2	640	558	15.4	101	nd
433	437	27.0	20.7	1.23	9.54	41.1	630	463	13.7	86.7	51.4
486	360	25.8	20.3	1.19	8.21	33.8	711	904	17.9	118	122
738	273	20.5	15.9	1.03	6.43	27.6	556	136	11.7	78.3	58.7
769	247	21.0	18.6	2.30	7.33	38.4	651	31.0	14.9	103	nd
Forest fire, controls											
1	50.5	14.6	34.3	2.00	3.26	45.6	487	1075	29.9	217	222
27	52.8	16.5	37.3	2.05	2.39	44.0	495	1243	30.9	216	310
56	51.9	14.5	40.5	2.56	4.02	47.0	501	1689	29.7	246	346
90	54.1	14.7	39.6	2.12	6.03	52.8	508	1446	26.1	252	322
343	96.0	16.0	33.2	1.58	2.78	33.4	447	1051	25.6	237	nd
374	95.8	16.4	34.2	1.83	3.56	36.5	492	1181	29.8	215	nd
399	90.4	14.5	36.9	1.99	5.64	57.9	485	1293	27.0	278	317
452	101.0	15.0	37.4	1.83	3.34	61.2	482	1968	28.0	254	294
704	87.9	14.6	36.7	2.37	3.01	43.4	489	1491	32.8	266	258
762	82.3	13.1	31.8	1.91	4.87	48.4	465	1060	29.9	240	nd
Forest fire, burned											
1	74.7	21.8	69.9	2.66	6.28	60.5	466	1551	22.1	170	187
27	88.1	22.9	46.7	1.92	1.24	29.8	435	1049	22.0	161	190
56	85.2	18.6	45.7	2.11	2.57	33.7	476	1313	19.6	161	165
90	85.9	19.9	41.2	1.95	2.81	35.1	478	945	22.6	178	158
343	128	20.2	32.6	1.65	2.84	26.3	433	741	24.5	162	nd
374	136	21.2	32.7	1.70	1.60	26.0	452	702	26.1	154	nd
399	124	19.9	26.9	1.40	3.46	31.2	434	747	22.3	174	173
452	124	20.2	27.7	1.68	2.17	22.4	436	841	23.9	163	223
704	114	19.3	23.6	1.99	1.47	12.5	437	560	24.8	155	187
762	112	18.0	21.0	1.98	2.66	22.2	411	542	25.2	159	nd

NOTE: Extr., extractable; nd, not determined.

Paper II

Fritze, H., Pennanen, T. and Pietikäinen, J. 1993. Recovery of soil microbial biomass and activity from prescribed burning. *Canadian Journal of Forest Research* 23: 1286-1290.



Recovery of soil microbial biomass and activity from prescribed burning

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Development of humus layer soil microbial biomass C (C_{mic}) and N (N_{mic}), fungal biomass (as soil ergosterol content), microbial respiration activity, and the soil organic C (C_{org}) and N (N_{tot}) were determined in coniferous forest soils that had received a single prescribed fire treatment at different times over a period of 45 years. The ratio of soil respiration rate to microbial biomass C (qCO_2) and the C_{mic}/C_{org} and N_{mic}/N_{tot} percentages were derived from the measurements taken. All the measured biomass indicators reacted identically to show recovery from prescribed burning within 12 years. A raised metabolic quotient (qCO_2) was detected in soils over the first 2 years following the fire treatment, but after the third year it had decreased to a stable level. These observations suggest that during the first few years after fire the soil microflora can be characterized on the basis of simple substrate–decomposer relationships. The first 12 years were characterized by increasing C_{mic}/C_{org} and N_{mic}/N_{tot} percentages, which then stabilized at mean values of 1.3 and 5.5%, respectively. The observed rise in the C_{mic} within a large pool of C_{org} suggested increasing availability of energy-rich C sources. These C sources are probably derived from the organic C input resulting from postfire plant succession.

FRITZE, H., PENNANEN, T., et PIETIKÄINEN, J. 1993. Recovery of soil microbial biomass and activity from prescribed burning. Can. J. For. Res. **23** : 1286–1290.

L'évolution de la quantité de C (C_{mic}) et de N (N_{mic}) dans la biomasse microbienne de la couche d'humus du sol, de la biomasse fongique (sous la forme du contenu en ergostérol du sol), de la respiration microbienne, et de la quantité de C (C_{org}) et de N (N_{org}) organique dans le sol, a été mesurée dans le sol de forêts de conifères soumises à un seul brûlage dirigé, à différents moments au cours d'une période de 45 ans. Le quotient métabolique (qCO_2) et les pourcentages de C_{mic}/C_{org} et N_{mic}/N_{org} ont été calculés à partir des données mesurées. Tous les indicateurs de biomasse mesurés ont réagi de la même façon et indiquaient un retour à la normale en dedans de 12 ans après le moment où le brûlage dirigé avait en lieu. Une hausse du quotient métabolique (qCO_2) a été observée dans les sols pendant les 2 premières années après le brûlage. Après la troisième année par contre, le quotient métabolique était revenu à un niveau stable. Ces observations suggèrent que, pendant les premières années après le feu, la microflore du sol peut être caractérisée sur la base d'une simple relation substrat–décomposeur. Les premiers 12 ans ont été caractérisés par une augmentation des pourcentages C_{mic}/C_{org} et N_{mic}/N_{org} qui se sont par la suite stabilisés à des valeurs moyennes respectives de 1,3 et 5,5%. Une hausse de la quantité de C_{mic}, telle qu'observée, dans un pool important de C_{org} suggère une disponibilité croissante de sources d'énergie riches en C. Ces sources de C provenaient probablement de l'apport de C organique résultant de la succession végétale après feu.

[Traduit par la rédaction]

Introduction

Lime has often been used to counteract anthropogenic soil acidification in coniferous forest soils. Other measures include wood ash fertilization and prescribed fire treatments. In the latter treatment aboveground vegetation is normally completely destroyed but species with subterranean regenerative organs may survive (Viro 1969). Combustion of the organic matter (mostly logging slash, live mosses, other surface vegetation, and the upper part of the humus F–H layer) releases mineral substances in the form of oxides which are readily turned into basic carbonates and hydroxides, thus leading to a decrease in acidity of the humus layer (Viro 1969). Soil microorganisms are key components in the biogeochemical cycling of various chemical elements, and hence of prime importance in maintaining the fertility of terrestrial habitats. Consequently, factors altering the rates of microbial processes in soil may be of importance for forest productivity. The soil microbial biomass acts as a sink for nutrients. In most arable soils the microbial biomass C and N, respectively, represent about 1–3% of the soil organic C (Anderson and Domsch 1989) and 2–6% of total soil N (Brookes et al. 1985). The values for coniferous and deciduous forest soils fall within the same range (Vance et al. 1987; Martikainen and Palojärvi 1990). Prescribed fire has been reported to induce decreases in soil microbial biomass C and N followed by a lack of recovery to control values within the first 3 years (Pietikäinen and Fritze 1993).

Two types of experimental design can be used to examine the recovery of the microbial biomass: (*i*) pre- and continued post-burning sampling from the same plot or (*ii*) sampling of time series postburned plots. The purpose of this study was to investigate the recovery of microbial biomass C and N, the fungal biomass (measured as the soil ergosterol content), basal respiration, and the metabolic quotient (qCO_2) in coniferous forest humus layer after prescribed fire by sampling time series plots. qCO_2 represents the amount of respired CO_2 per unit microbial biomass C in a nonamended soil (no extra substrate given). Data concerning these figures are relatively sparse for coniferous forests and therefore their estimation improves the understanding of nutrient cycling in these ecosystems.

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Study area

The study was conducted in southern Finland at Evo (61°12′N, 25°07′E), where there is a long tradition of using fire in forestry practice. The plots were restricted to a small study area containing rather homogenous soils (cf. Lindholm and Vasander 1987). Study plots were located in forest stands that had received a single prescribed fire treatment at different times. The vegetation and stand development of forests after fire treatment have been described for this area by Lindholm and Vasander (1987). In brief, Norway spruce (*Picea abies)* dominated *Myrtillus* site type (Cajander 1949) forests were clear-cut, and the logging slash was burned the following year. The area was then seeded with Scots pine (*Pinus sylvestris*). Lindholm and Vasander (1987) described three postfire successional communities: young (0–3 years after burning), open older (5–8 years after burning), and young forested (14–27 years after burning) sites. In the first group typical plant species were *Marchantia polymorpha*, *Epilobium collinum*, *Viola rupestris*, *Rubus idaeus*, *Vicia sylvatica*, *Rumex acetosa*, *Ceratodon purpureus*, *Carex digitata*, and *Luzula pilosa*. The second group was characterized by the species *Calluna vulgaris*, *Vaccinium vitis-idaea*, and *Cladonia* spp., giving this group a more xerophilic vegetation pattern. The vegetation of the third group was also characterized by an increased proportion of grasses and herbs and by plants normally found in mature forest, e.g., *Vaccinium myrtillus*, *Dicranum polysetum*, and *Pleurozium schreberi*.

Materials and methods

Study plots, soil sampling, and treatment

Sixteen plots on till that had been burned between 0 and 45 years earlier were sampled in June and August of 1991 (Table 1). The tree stand at study plot 15 was fertilized in 1983 with an N–P fertilizer (600 kg·ha^{-1}) containing 26% NH_4NO_3-N and 14% P_2O_5-P. The tree stand at plot 16 was fertilized with 550 kg·ha^{-1} urea and NH_4NO_3 in 1977. Both stands were also thinned. At each study plot 10 separate soil samples (soil core diameter 7.2 cm) were taken from the entire humus horizon (F–H) and combined to give a single bulk sample per plot, as described by Fritze (1991). The sample was sieved to pass a 4-mm mesh and stored at 4°C. Subsamples of the humus were oven-dried at 105°C for 12 h to determine the dry weight and subsequently heated to 550°C for a minimum of 3 h to determine the organic matter as loss on ignition. Sample density was determined by weighing 5-cm^3 aliquots of dried homogenized soil and used for the microbial biomass determination. Humus total C (C_{tot}) and N (N_{tot}) content was measured with a Leco CHN-900 analyzer in duplicate. C_{tot} in this study is synonymous with C_{org}, since organic C is the predominant form present. The amount of inorganic C (mainly carbonates) in an acid forest humus on till was negligible except for the first two plots (0 and 1 year after fire), where due to the higher pH, carbonates were expected and therefore included in the C_{tot} measurement. The pH was determined in soil–water suspensions (w/v, 1:10). To convert the measurements to a hectare basis, the thickness of the humus layer and the adjusted density, D_f (Macadam 1987), of the samples were determined once in August 1991 from 10 measurements per plot.

Biological determinations

The production of CO_2 (basal respiration) was measured by gas chromatography (Fritze et al. 1989) using 2.5 g of fresh humus. Samples were adjusted to 60% of the water holding capacity and preincubated for 5 days at 14°C in a 120-mL glass bottle. Measurements of capped bottles were made 50 h after the start of incubation. Three replicates were made for each analysis.

Microbial biomass C (C_{mic}) and N (N_{mic}) were determined by the fumigation–extraction method (Vance et al. 1987). The 0.5 M K_2SO_4 extractable C and N from the chloroform-fumigated samples and their unfumigated controls were converted to microbial C and N using the sample density and the equation described by Martikainen and Palojärvi (1990): $C_{mic} = (1.30\ C_f + 309)\ \mu g \cdot cm^{-3}$ and $N_{mic} = (1.38N_f + 45.3)\ \mu g \cdot cm^{-3}$ for the biomass C and N respectively, where C_f and N_f are the carbon and nitrogen (fumigated minus controls) measured from the extracts. Three replicates were made for each analysis. The extractable C was determined on a Shimadzu TOC-5000 total organic carbon analyzer. Total N of the extract was determined by the Kjeldahl method.

Fungal biomass was measured with the ergosterol method (Grant and West 1986). Ergosterol extraction was as follows. Fresh soil (0.25 g wet wt.), containing 7-dehydrocholesterol (50 μL of a 0.91 μg·μL^{-1} solution in methanol) as an internal standard, was extracted with 2 mL of methanol, vortexed, and centrifuged for 10 min at 3000 rpm. The supernatant was removed, and the remaining soil was washed twice with the same volume of methanol. To the combined methanol supernatants, 0.2 mL 4% KOH (in 96% ethanol) per millilitre of methanol was added, and the solution was incubated for 30 min at 80°C. Distilled water and hexane (2 mL of each) were then added, and the hexane was phase separated. After a repeated hexane extraction the combined hexane phases were dried under nitrogen. The extracted sterol material was dissolved in 500 μL acetonitrile (CH_3CN; Fissons FSA supplies, Sigma) and analyzed by high-pressure liquid chromatography (HPLC). The HPLC was equipped with a LiChrosorb RP C 18 column, and ergosterol was detected at 282 nm. Hexane–isopropanol–acetonitrile (5:5:90) was used as a carrier. All chemical solvents were of HPLC grade. The determination was performed on all the soil samples without replicates, but repeated within 2 days.

For the calculation of the qCO_2 (μL CO_2·mg C_{mic}^{-1}·h^{-1}) the hourly mean of CO_2 output was divided by the unit biomass C of the respective sample. The C_{mic}/C_{org} and the N_{mic}/N_{tot} ratios were calculated by dividing the microbial biomass C and N with the respective soil C_{org} and N_{tot} and presented as a percentage.

Statistical analysis

The data were normally distributed as assessed by the Wilk–Shapiro test. Analysis of variance (ANOVA) was used to test the difference between the two samplings. Correlation analysis to the biomass measurements was performed. The Statistix 3.1 (NH Analytical Software) statistical package was used.

Results

Results are presented on a soil organic matter (loss on ignition) basis as means of the two samplings (Table 1). The two samplings did not differ (ANOVA) in respect to the amounts of soil total N ($F = 2.33$; $p > 0.05$) and C ($F = 3.99$; $p > 0.05$). The factor for converting the results to a soil dry weight basis (organic matter percentage, Table 1) differed significantly between the samplings.

The amount of humus N and C and the derived C/N ratio did not show any time trends, unlike pH (Table 1), which declined from 6.0 to around 4.4 within a time span of 10 years after the start of the succession. The pH between the two samplings did not differ significantly ($F = 1.67$, $p > 0.05$).

The basal respiration (Fig. 1), the amount of microbial biomass C and N (Table 1), and the fungal biomass (measured as ergosterol) (Fig. 2) showed similar reactions. All measured variables showed a decrease towards minimum levels during the first 2 years from the start of the plant succession; rising then towards a plateau at 12 years, and then declining again 40 years after fire. Microbial biomass did not differ between the samplings ($F = 0.00$ and 0.17, $p > 0.05$; for both microbial biomass C and N, respectively), whereas the respiration from the June measurement were higher values ($F = 5.26$, $p < 0.05$) than those from the August measurement. The ergosterol measurement was performed only once from the June sampling. The correlation between biomass C and N was high ($r = 0.933$) as were the correlations between biomass C and the fungal ergosterol ($r = 0.824$) and biomass N and ergosterol ($r = 0.693$).

The C_{mic}/C_{org} and N_{mic}/N_{tot} percentages did not differ between the samplings ($F = 0.62$ and 0.15, $p > 0.05$, respectively) and

TABLE 1. Characteristics of the study plots

Study plot	Years after fire	Humus layer (cm)	Tree height (m)	Fl[a]	N_{tot} ($mg \cdot g^{-1}$)	C_{org} ($mg \cdot g^{-1}$)	OM[b] (%)	pH	MBC[c] ($\mu g \cdot g^{-1}$)	MBN[d] ($\mu g \cdot g^{-1}$)
1	0	3.2	0	25.6	20.2±0.55	539±10.4	72.5±13.8	5.96±0.29	4424±637	643±29.9
2	1	7.0	0	49.0	20.4±0.86	545±16.2	80.0±5.06	5.76±0.18	3299±208	510±68.3
3	2	4.1	0.3	42.9	19.8±0.52	536±7.31	74.5±7.45	5.09±0.08	2772±3.99	381±15.6
4	5	2.5	0.5	15.0	17.8±2.35	527±85.0	62.4±8.27	4.69±0.04	3961±174	639±52.7
5	9	3.9	1–2	40.7	18.4±1.03	510±23.7	76.8±3.41	4.37±0.27	4314±1.21	718±79.7
6	12	2.8	2–3	30.7	29.7±1.56	742±60.0	43.4±4.84	4.59±0.08	7985±401	1253±145
7	13	3.0	3–4	14.7	15.8±1.69	445±39.3	57.3±12.1	4.36±0.08	6615±324	996±69.1
8	14	4.7	3.5–4.5	46.9	17.8±0.78	529±15.8	79.7±7.64	4.32±0.17	5580±234	844±90.1
9	15	4.2	3.5–4.5	37.9	16.5±1.73	484±21.8	64.5±0.24	4.34±0.06	6516±85.2	935±8.60
10	17	3.2	4.5–6.0	22.0	21.8±0.80	544±6.04	45.4±8.19	4.79±0.12	7858±398	1197±17.9
11	23	7.2	6.0–8.0	40.8	19.8±0.66	547±15.3	69.5±3.79	4.13±0.11	6555±285	937±32.1
12	28	4.8	8.0–10	29.0	22.3±2.91	584±76.5	39.6±0.52	4.62±0.14	7687±912	1076±32.4
13	36	3.6	10–13	30.9	21.7±2.58	490±13.9	51.9±10.5	4.67±0.09	7256±114	1315±63.8
14	39	4.9	10–15	33.2	20.1±0.41	527±14.1	59.6±9.06	4.38±0.12	6296±155	981±57.7
15	43	5.6	10–15	43.0	18.2±2.63	559±74.1	72.2±8.10	3.90±0.15	6038±708	813±80.5
16	45	4.5	10–15	48.8	23.2±3.37	650±86.4	57.6±4.92	4.25±0.16	5637±28.4	908±41.1

NOTE: The values are the means of the two samplings and their SE are presented. For N_{tot}, C_{org}, MBC, and MBN, organic matter was measured as loss on ignition.
[a] Conversion factor (10^3 kg·ha^{-1} (organic matter measured as loss on ignition)) to recalculate the sampling results to a hectare basis.
[b] Organic matter measured as loss on ignition given in percentage of ovendried soil.
[c] Microbial biomass C.
[d] Microbial biomass N.

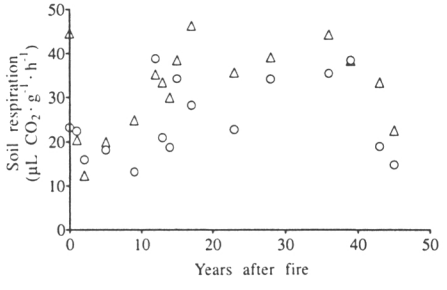

FIG. 1. Development of the soil microbial activity, measured as soil respiration, after prescribed fire. The values are means of three replicate measurements made on the first (△) and second (○) sampling, respectively. Organic matter was measured as loss on ignition.

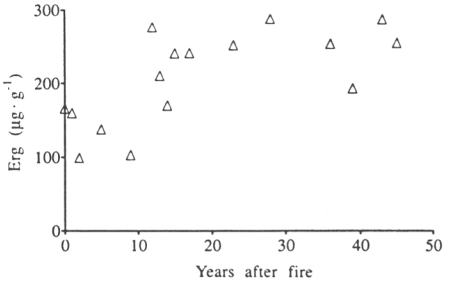

FIG. 2. Development of the fungal biomass, measured as the soil ergosterol content (Erg), after prescribed fire. The values are means of two replicate measurements. Symbols as in Fig. 1. Organic matter was measured as loss on ignition.

increased during the first 12 years of succession. After the 12th year the C_{mic}/C_{org} percentage showed no clear change, but varied around a mean value of 1.3%, except for a drop in the two oldest plots (Fig. 3*a*). The N_{mic}/N_{tot} percentage reacted identically (Fig. 3*b*) with the C_{mic}/C_{org} percentage, levelling at a mean value of 5.5% within the same time span. Higher qCO_2 values were detected immediately after the fire treatment (Fig. 4), which then declined to nearly constant mean values. The two oldest plots showed a declining qCO_2. The quotient differed between the two samplings ($F = 8.80$, $p < 0.05$), but reacted similarly.

Discussion

The soil microbial biomass acts as a sink for important nutrients needed also for plant growth. It is thus important to know the response of the microbial biomass to different treatments. Prescribed burning decreases microbial biomass (Pietikäinen and Fritze 1993). Microbial biomass C represents about 1–3% of the soil organic C in arable soils (Anderson and Domsch 1989), and the proportion of the microbial biomass N out of the soil total N is within the range 2–6% (Brookes et al. 1985). Martikainen and Palojärvi (1990) report C_{mic}/C_{org} and N_{mic}/N_{tot} percentages of 1.19 and 5.94%, respectively, based on estimations from four different forest stands. In this study the C_{mic}/C_{org} and N_{mic}/N_{tot} percentages increased until the 12th year after the fire, reaching mean values of 1.3 and 5.5%, respectively. It is known that free ammonia increases in the soil after prescribed fire (Viro 1969), and this was also observed in this study (data not shown). The N_{mic}/N_{tot} percentage suggests that the microbial biomass is not a competitor for ammonia over a certain time span after a fire. The increasing percentages are due to the recovery of the microbial biomass after fire and not to different soil C_{org} or N_{tot} levels. The observed rise in the C_{mic} within a large pool of C_{org} indicates increasing availability of energy-rich C sources. These C sources probably derive from the organic C input of the postfire plant succession.

Insam and Domsch (1988) defined the usefulness of estimating the C_{mic}/C_{org} ratio as follows: the basic carbon and energy source for heterotrophic production is the carbon input

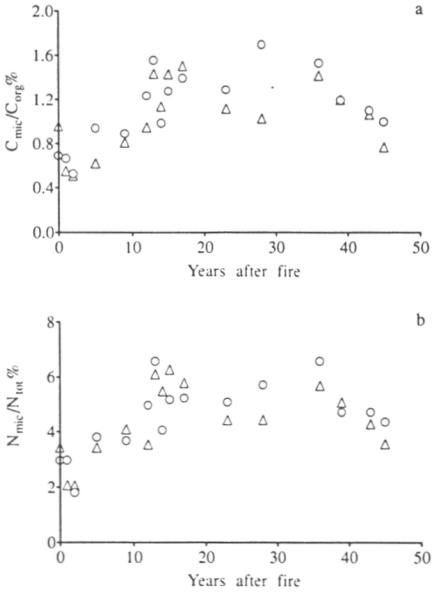

FIG. 3. Development of (*a*) the C_{mic}/C_{org} percentage and (*b*) the N_{mic}/N_{tot} percentage after prescribed fire. Symbols as in Fig. 1.

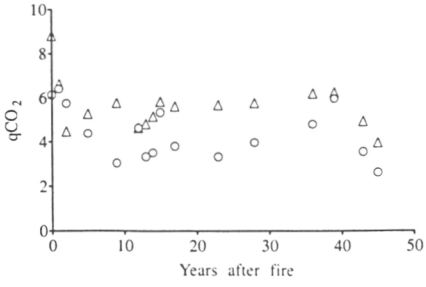

FIG. 4. Development of the metabolic quotient (qCO_2) after prescribed fire. Symbols as in Fig. 1.

from net primary production (NPP). As long as NPP exceeds the respiration of heterotrophs (R) in any ecosystem, organic matter will accumulate; as soon as R equals NPP a steady state will be reached. Agricultural ecosystems in equilibrium are characterized by a constant C_{mic}/C_{org} ratio (Anderson and Domsch 1986), which is not universal (Anderson and Domsch 1989), but has to be determined for each ecosystem. It is also hypothesized that soils exhibiting a C_{mic}/C_{org} ratio deviating from their constant would be either losing or accumulating C (Anderson and Domsch 1986). Thus, if the equilibrium constant is known, the actual C_{mic}/C_{org} ratio of a disturbed soil should provide information on how near the soil is to its equilibrium state (Insam and Domsch 1988). Anderson and Domsch (1986) hypothesized that in agricultural soils under steady-state conditions, with a constant C_{mic}/C_{org} percentage, the C_{mic} would represent 40% of the annual C input to the ecosystem. If we assume that a mean C_{mic}/C_{org} value of 1.3% is representative of young pine stands we can calculate the mean annual C input into this ecosystem according to the hypothesis of Anderson and Domsch (1986) by knowing either C_{mic} or C_{org}. For the 28-year-old pine stand (plot 12; conversion factors used are given in Table 1) this would mean an annual C input of 550 kg/ha. Mälkönen (1974) calculated the annual aboveground biomass C entering the soil ecosystem in a 28-year-old pine stand to be around 1000 kg/ha. The annual belowground biomass C input was not calculated. The conclusion from these two values is that in the coniferous forest ecosystem the C_{mic} seems not to represent 40% of the annual input of C. This calls for an empirical investigation of the C_{mic}/C_{org} percentage in different coniferous forest ecosystems in relation to the annual C input.

qCO_2 was highest immediately following burning, declining to a stable level in the humus layer from the third year onwards. Higher qCO_2 values are characteristic for young topsoil layers in a soil chronosequence (Insam and Domsch 1988; Insam and Haselwandter 1989) and can be related to ecosystem succession. Insam and Haselwandter (1989) discussed that the change in $\bar{q}CO_2$ is related to simple substrate–decomposer relationships dominated by fast-growing r strategists. With progressing succession, when detritus food webs become more complex, slower growing specialists, the K strategists, occupy various niches. This could mean that in the first 2 years after the fire the microbial species composition in the soil is different from the species composition of the soils taken from the fifth year onwards. But the observed change in the qCO_2 could also be due to changed substrate quality after fire.

There is a drop of the measured biological variables at the two oldest plots (15 and 16) to be noted. Two reasons for this observation can be given: (*i*) the stands were thinned, years before we took the soil samples, and this could decrease the annual C input; (*ii*) the stands were heavily fertilized with N fertilizers. Urea or NH_4NO_3 application to coniferous forest soil in the range used for plots 15 and 16 is known to reduce the microbial biomass C (Nohrstedt et al. 1989).

In conclusion, it takes the microbial biomass C and N at least 10 years from the fire treatment to recover to values reported in the literature. The reactions of the microbial biomass measurement closely correlated with that of the fungal biomass. This implies that the fungal biomass represents a high proportion of the soil microbial biomass in coniferous forest when analyzed by the two biomass methods.

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Paper III

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CLEAR-CUTTING AND PRESCRIBED BURNING IN CONIFEROUS FOREST: COMPARISON OF EFFECTS ON SOIL FUNGAL AND TOTAL MICROBIAL BIOMASS, RESPIRATION ACTIVITY AND NITRIFICATION

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Summary—The effects of clear-cutting (CC) and clear-cutting followed by prescribed burning (CC-B) on humus chemical and microbiological variables and quality were compared in a Norway spruce dominated stand in North-Eastern Finland. The pattern of chemical changes in humus was similar after both treatments but CC-B caused greater changes than CC. Treatments raised the pH, cation exchange capacity and base saturation compared to an untreated standing forest control (Ctr). Total microbial carbon (C_{mic}) measured by substrate-induced respiration (SIR) and fumigation–extraction (FE) methods decreased following treatments. CC caused a 21% reduction of C_{mic} compared to Ctr (10,890 $\mu g\ g^{-1}$ dry wt), as measured by SIR, and a 27% reduction compared to Ctr (7281 $\mu g\ g^{-1}$ dry wt) as measured by FE. CC-B resulted in 53 and 67% lower C_{mic} than Ctr as measured by SIR and FE, respectively. Reasons for this decline in C_{mic} are proposed. Fungal biomass determined as humus ergosterol concentration fell even more steeply than total C_{mic}. Humus quality was analysed by near infrared reflectance spectroscopy (NIR) which revealed differences in humus structure between treatments. The NIR data could be interpreted to explain 75–82% of the variation in C_{mic}-FE, C_{mic}-SIR and ergosterol concentration. CC and CC-B lowered soil basal respiration, but not proportionally with the reduction in C_{mic} since the specific respiration rate (CO_2-C evolved per unit C_{mic}) was clearly higher with CC-B than CC or Ctr. CC and CC-B both resulted in a higher concentration of NH_4^+ but only the humus from CC-B showed nitrification during a 6 week laboratory incubation.

INTRODUCTION

In boreal coniferous forests, prescribed burning is used in preparing areas for forest regeneration after timber harvesting. As burning raises soil pH and cation concentrations (Viro, 1974; Macadam, 1987), it can be used to prevent soil acidification. Soil microorganisms are the key components in forest litter decomposition and thus account for the cycling of nutrients. The microbial pool in soil is of major important in maintaining site fertility. In coniferous forest soils, microbial biomass carbon (C_{mic}) represents *ca* 1.2% of soil total organic carbon (Martikainen and Palojärvi, 1990; Fritze *et al.*, 1993). Burning causes a profound reduction in soil C_{mic}, the recovery from which may take as long as 12 yr (Fritze *et al.*, 1993).

Prescribed burning as a forestry practice in Scandinavia involves a combination of three processes: (1) clear-cutting; (2) heat treatment during burning; and (3) ash deposition from burned slash and humus. All three factors could cause the reduction in C_{mic} and respiration activity after burning either individually or in combination. Clear-cutting has been shown to reduce soil fungal biomass in humus and mineral soil, which is probably due to the cessation of root growth and exudation (Bååth, 1980). High temperatures during burning affect soil microbial biomass (Dunn *et al.*, 1985), but the intensity of the heat effect is determined by the amount of slash burned and the insulating property of the humus layer (Vasander and Lindholm, 1985). In a chemical respect, the spreading of wood ash on the intact humus layer resembles the effects of burning, since wood ash contains high amounts of basic cations and thus raises pH and liberates nutrients. In an uncut forest stand, wood ash application of 1000 $kg\ ha^{-1}$ raised pH and the degree of base saturation to the same extent as burning, but had no effect on the amount of fungal biomass or total C_{mic} (Fritze *et al.*, 1994b). In contrast to burning, wood ash application does not result in reduction of C_{mic} in soil. Consequently, the reduction in C_{mic} following burning is caused either by clear-cutting or a heat-induced effect or a combination of both.

We compared the effects of clear-cutting and burning on soil microbial biomass, concentration of fungal ergosterol and respiration and nitrification activities. In addition to conventional chemical characterization, we used near infrared reflectance (NIR) analysis to provide a fingerprint of the quality of the humus following treatments. The randomized

block design used in our study enabled us to separate the effects of the individual treatments.

MATERIALS AND METHODS

Study area

The experimental area is located in Suomussalmi, northern Finland (65° 15′ N, 28° 50′ E) at an elevation of 280 m a.s.l. The soil type is podzol on moraine. The experimental area was covered by a mixed forest of Norway spruce [*Picea abies* (L.) Karst.] with some 200–300 yr old Scots pines [*Pinus sylvestris* (L.)]. According to the Finnish forest type classification, the area represented Vaccinium–Myrtillus type (VMT). Two different forest regeneration practices were compared in this random block designed experiment. The treatments were clear-cutting (CC) and clear-cutting followed by prescribed burning (CC-B); a control (Ctr) plot with untreated standing forest was included in each block of treatments. The treatments were performed on 50 × 50 m plots and replicated in four blocks. The CC- and CC-B-plots were logged in June 1990 and the logging residue was left on the ground. The CC-B-plots were burned on 3 June 1992.

Sampling and analyses for moisture, organic matter and pH

Humus samples were taken from the F/H horizon using a 72 mm dia soil corer on 2 June 1993. From each plot, 20 humus cores were collected and combined to form one bulk sample. Three treatments (CC, CC-B and Ctr) in four blocks resulted in 12 composite samples. At the time of sampling, 3 yr had elapsed since clear-cutting and 1 yr since prescribed burning. After sampling, humus was sieved (2.5 mm) and stored at 4°C. Humus dry weight was determined after drying triplicate subsamples at 105°C overnight. Dry weight of air-dried samples was determined in the same way and used to correct the data from chemical analyses on a dry weight basis. Soil organic matter was determined from the dried samples as loss on ignition after ashing at 550°C for 4 h. pH was measured in humus–water suspensions (15 cm^3 humus plus 25 ml water).

Chemical analyses

For determining cation concentrations and exchangeable acidity, 15 cm^3 (weight recorded) of air-dried and homogenized humus subsamples were extracted in 150 ml 0.1 M $BaCl_2$ solution (unbuffered). The humus suspensions were left to stand overnight, then shaken for 1 h and filtered. 100 ml of the filtrate was used to measure the concentrations of Ca, Mg, K and Na by an inductively-coupled plasma-emission spectrometer (model 3580, ARL). The remaining 50 ml was titrated to pH 7 with 50 mM NaOH, the amount of base (expressed in cmol kg^{-1} humus dry wt) used in titration indicated the exchangeable acidity of humus. The cation exchange capacity (CEC) was calculated as the sum of Ca, Mg, K and Na (cmol positive charge kg^{-1} humus dry wt) plus exchangeable acidity. The percentage of Ca, Mg, K and Na of CEC indicates the degree of base saturation (BS) in humus. Total N and organic C were determined by dry combustion (CHN-600, Leco Corp.) after removing any possible carbonates by adding acid.

Near infrared reflectance spectra

The near infrared reflectance (NIR) spectra of the freeze-dried and homogenized humus samples were measured with a Perkin–Elmer System 2000 FT-IR instrument set to scan the humus samples from 11,000 to 3000 cm^{-1}. The diffuse reflectance technique (DRIFT) was used in all measurements (Palmborg and Nordgren, 1993; Fritze *et al.*, 1994a).

Biological determinations

The biological determinations were carried out within 14 days after sampling. Microbial biomass was determined by the chloroform fumigation–extraction (FE) method (Vance *et al.*, 1987) using duplicate samples of fresh humus equalling 2 g dry wt. The fumigated and non-fumigated control samples were extracted with 45 ml 0.5 M K_2SO_4 and shaken (200 rev min^{-1}) for 30 min (Martikainen and Palojärvi, 1990). Suspensions were filtrated through 0.22 μm filters (Millipore) before the determination of soluble organic carbon on a Shimadzu TOC-5000 total organic carbon analyzer. Microbial biomass carbon (C_{mic}; μg C g^{-1} humus dry wt) was calculated by the linear regression model derived from Martikainen and Palojärvi (1990): C_{mic} = (C from fumigated samples − C from non-fumigated samples) · 1.9 + 428.

The substrate induced respiration (SIR) method (Anderson and Domsch, 1978) was used to determine the metabolically active proportion of C_{mic}. Fresh humus samples equalling 2 g dry wt were weighed into 125 ml glass bottles and placed in a water bath at 22°C. Samples were moistened with glucose solution plus water to give a soil moisture content of 60% of water holding capacity (WHC) and a glucose concentration of 20 mg ml^{-1} soil water (Priha and Smolander, 1994). The samples were allowed to condition for 30 min, then the bottles were capped, incubated for 2 h and CO_2 evolved was measured on a Varian 3600 g.c. equipped with a TC detector and a Megabore GS-Q capillary column (J&W Scientific). Injector, column, and detector temperatures were 120, 30 and 150°C, respectively. Helium was used as carrier gas. C_{mic} (μg g^{-1} humus dry wt) was calculated from hourly respiration rate measurements according to Anderson and Domsch (1978): $C_{mic} = 40.04 \cdot y + 3.7$, where y = ml CO_2 h^{-1} g^{-1} soil dry wt. Soil basal respiration was measured by g.c. (see above) on 2.5 g samples of fresh humus as CO_2 evolved in 48 h (μl CO_2 g^{-1} humus dry wt). Basal respiration was measured both on samples adjusted

to 60% of WHC and on field moist samples. The metabolic quotient, qCO_2 ($\cdot 10^{-3}\,h^{-1}$), is defined as the mass of CO_2 -carbon evolved h^{-1} mass C_{mic}^{-1}, and was calculated by dividing the hourly respiration rate (in $\mu g\ CO_2 - C\ g^{-1}$ humus dry wt) of humus adjusted to 60% of WHC by the corresponding C_{mic} (mg C g^{-1} humus dry wt) determined by the FE-method. Basal respiration rate was additionally measured from samples adjusted to 60% of WHC after a 6 week incubation at 14°C. The same samples were used for studying nitrogen transformations (see below).

Nitrification capacity of the humus was determined in a pot experiment, where fresh humus equalling 2 g dry wt was placed in 125 ml bottles, moistened to 60% of WHC and incubated in the dark at 14°C for 6 weeks. During the incubation, the moisture content of the samples was adjusted on three occasions. After 6 weeks, the incubated samples and their unincubated frozen controls were extracted with 40 ml 1 M KCl. The humus suspensions were shaken for 2 h at 200 rev min^{-1} and filtered through Schleicher and Schuell 589^3 -filter paper. Solutions were analysed colorimetrically for NH_4^+ and NO_3^- by a flow injection analyser (Tecator FIA Star 5020 and spectrophotometer 5023).

Fungal biomass was measured as humus ergosterol content (modified from Grant and West, 1986). For ergosterol extraction, 0.25 g wet wt humus, with 7-dehydrocholesterol added (45.5 μg in 50 μl methanol) as internal standard, was extracted with 2 ml methanol, vortexed, and centrifuged for 10 min at 3000 rev min^{-1}. The supernatant was removed and the pellet re-extracted twice by the same procedure. The supernatants from all steps were combined and 1.12 ml 4% KOH in ethanol was added. The solutions were reacted for 30 min at 80°C, cooled, 2 ml distilled water and 2 ml hexane added and the sample mixed. The hexane phase was allowed to separate and was removed. The hexane extraction was repeated with a further 2 ml of hexane and the combined hexane phases were evaporated under a stream of N_2. The dry sterol residue was dissolved in 1 ml acetonitrile and analysed by HPLC (L-4250 U.V.-VIS. Detector, L-6200 Pump, Merck Hitachi) using a LiChrosorb RP C 18 column. Ergosterol and internal standard were detected at 282 nm. Hexane–isopropanol–acetonitrile (5:5:90 v/v/v) was used as eluent. All solvents were of HPLC quality. Pure egosterol and internal standard samples were also analysed, the percent recovery of the internal standard in the humus sample was calculated and used to determine the concentration of ergosterol.

Statistical analyses

All the results are expressed on an oven-dry basis and are the means of the treatments in the four separate blocks. 2-way analysis of variance (ANOVA) followed by Tukey's test was performed on the data to detect the effect of the treatments and the blocks. The pH results had to be $\log_{10}$ transformed to achieve equal variances. A correlation analysis between the C_{mic} values derived by FE and SIR was performed. The Statistix 4.0 (NH Analytical software) statistical program was used.

The spectral NIR (% transmittance) data were subjected to principal component analysis (PCA) to elucidate major variation and covariation patterns. The following pretreatment was made to the NIR data: the 8000 wavenumber points of the spectra were reduced to 1000 and multiple scatter corrected between wavenumbers 10992 and 7800. In the PCA, the NIR wavenumbers between 3808 and 3000 cm^{-1} were deleted in order to remove outliers. The NIR data were subjected, together with the biological data, to partial least squares (PLS) regression. PLS performs a simultaneous and interdependent PCA decomposition in both X- and Y-matrices in such a way that the information in the Y-matrix is used directly as a guide for optimal decomposition of the X-matrix. The biological measurements were read into the Y-matrix and the NIR spectra into the X-matrix. The percentage of the variance of the biological data explained by the first component of the spectral data was calculated.

The PLS model of Fritze *et al.* (1994a) constructed for the humus NIR spectra in the X-matrix and the microbial biomass (C_{mic}-FE) in the Y-matrix from wood ash fertilization and burning test areas was used to predict the C_{mic} of this study from the measured NIR data. Before modelling, the following additional (see above) manipulations were performed on the data: the NIR data normalized and $\log_{10}$ C_{mic} values were used. The Unscrambler II program (CAMO A/S) was used for the multivariate statistical analysis.

RESULTS

Nutrients and acidity

The concentration of Ca in humus was the variable most affected by the treatments: CC alone caused a 1.3-fold-increase in Ca compared to Ctr and CC-B resulted in a further 2.5-fold-increase in the concentration of Ca of (Table 1). The trend in Mg followed the same pattern, but the detected differences were smaller: only the increase from the control concentration (Ctr: 394 $\mu g\ g^{-1}$ dry wt) to the postburn level (CC-B: 639 $\mu g\ g^{-1}$ dry wt) was significant. Both treatments, CC and CC-B, resulted in significant decrease in K and Na. The concentrations of these elements following the treatments were *ca* 50% of the control values (Ctr: 1150 and 62 $\mu g\ g^{-1}$ dry wt for K and Na, respectively). No differences between CC and CC-B in K and Na concentrations were detected. CC-B reduced soil organic matter and concentration of soil organic C. Neither CC nor CC-B treatment caused any change in total N content compared to Ctr. No statistically significant block effect was found in any of the measured physiochemical or biological variables.

Cation-exchange capacity (in cmol kg^{-1}) of the CC area (33.6) did not differ from that of the Ctr (32.3). CC-B caused a significant 50%-increase in CEC to 51.9. Due to the higher pH and greater concentration of Ca, the degree of base saturation in CC humus was clearly higher than in Ctr humus (69.0 and 61.7%, respectively) and CC-B raised BS still further to 97.6%. The pH value of 4.1 of the CC area compared to the Ctr pH of 3.9 was not significantly higher, but CC-B was clearly effective in raising the pH clearly by 1.7 units.

The humus quality was affected by the treatments applied. The humus NIR spectra of the different treatments from one representative study plot are shown as an illustration in Fig. 1. The PCA performed on the whole NIR data set separated the treatments along the first component, which explained 99.6% of the variation in the data set (Fig. 2).

Microbial biomass and activity

Both measures of total microbial carbon; C_{mic}-FE and C_{mic}-SIR showed decreasing trends following CC and CC-B (Table 1). Clear-cutting caused a significant reduction in C_{mic}-FE and humus ergosterol content. Burning reduced C_{mic} further, and the decrease was significant in all microbiological variables measured. If the amount of C_{mic} in Ctr humus of each determination is considered as 100%, the proportions of C_{mic} for CC and CC-B are 73 and 33% by FE and 79 and 47% by SIR, respectively. The fall in C_{mic} measured by FE is steeper than that indicated by SIR (Fig. 3). The C_{mic} values measured by FE ranged between 2023 and 8070 $\mu g\, g^{-1}$ dry wt and those obtained by SIR varied from 4604 to 12570 $\mu g\, g^{-1}$ dry wt. The correlation between C_{mic}-SIR and C_{mic}-FE was 0.95 ($P < 0.001$) but the values obtained by SIR were 50, 62 and 115% higher for Ctr, CC and CC-B respectively than those from FE.

Microbial activity measured by means of soil respiration is clearly affected by both CC and CC-B. Soil respiration was measured separately using three different variations of sample moisture and incubation time: (1) field moist samples measured at sampling time; (2) moistened (to 60% of WHC) samples measured at sampling time; and (3) moistened samples measured after 6 weeks at 14°C. All methods showed reduced respiratory activity following CC and CC-B, although not every reduction was statistically significant (Fig. 4). The metabolic quotient qCO_2 determining the specific respiration rate as CO_2-C evolved per unit C_{mic} (expressed as $\cdot 10^{-3}\, h^{-1}$) was clearly higher at CC-B (4.0) than at Ctr (2.3) or CC (2.4) sites.

By analysis of the spectral NIR data, 62, 79, 75 and 82% of the respective variations in soil basal respiration, ergosterol, C_{mic}-SIR and C_{mic}-FE could be explained by the first PLS component (Table 2). The microbial activity and amount of biomass was thus related to the quality of the humus.

Nitrification

Nitrification was determined as nitrate–nitrogen formed during a 6 week incubation (Table 3). Simultaneously, the concentration of ammonium–nitrogen was expected to diminish. The initial NO_3^--N and NH_4^+-N concentrations in Ctr humus were 0.66 and 46.7 $\mu g\, g^{-1}$ dry wt. The respective postincubation values were 0.54 and 30.0 $\mu g\, g^{-1}$ dry wt, which indicate no nitrification in Ctr humus. In CC and

Table 1. Treatment effects of the measured variables

	Control		Clear-cut		Clear-cut + burned	
Variable	Mean	SE	Mean	SE	Mean	SE
pH	3.93a	0.04	4.09a	0.08	5.85b	0.22
OM (%)	89.2a	0.23	87.8a,b	2.04	72.6b	6.06
C_{org}	486a	5.44	485a	10.9	449a	24.6
N_{total}	11.1a	0.47	11.5a	0.39	12.4a	0.24
Ca	2709a	158	3542b	233	8824c	134
Mg	394a	28.2	479a	26.5	639b	30.4
K	1150a	58.4	570b	20.8	494b	62.8
Na	61.9a	2.50	33.5b	4.06	29.5b	3.03
CEC	32.3a	0.98	33.6a	0.89	51.9b	0.47
BS (%)	61.7a	0.91	69.0b	2.33	97.6c	0.54
C_{mic}-FE	7281a	439	5293b	222	2398c	218
C_{mic}-SIR	10890a	719	8582a	516	5120b	343
Erg	246.9a	14.5	167.0b	3.41	73.5c	10.4
qCO_2	2.29a	0.11	2.43a	0.08	3.99b	0.29

Organic matter (OM %) is given in weight percentage of an oven dry soil (dry wt); C_{org} (organic carbon) and N_{total} (total nitrogen) are given in mg g^{-1} dry wt. Extractable cations (Ca, Mg, K, and Na) are given in $\mu g\, g^{-1}$ dry wt and the CEC (cation exchange capacity) and BS (base saturation) as cmol kg^{-1} dry wt and percentage of CEC, respectively. C_{mic}-FE (microbial biomass C measured by the fumigation–extraction method), C_{mic}-SIR (microbial biomass C measured by the SIR method), and Erg (ergosterol) are given in $\mu g\, g^{-1}$ dry wt. The unit of the metabolic quotient qCO_2 is $\times 10^{-3}\, h^{-1}$. Means followed by the same letter are not significantly different at the $P < 0.05$ level (Tukey's test).

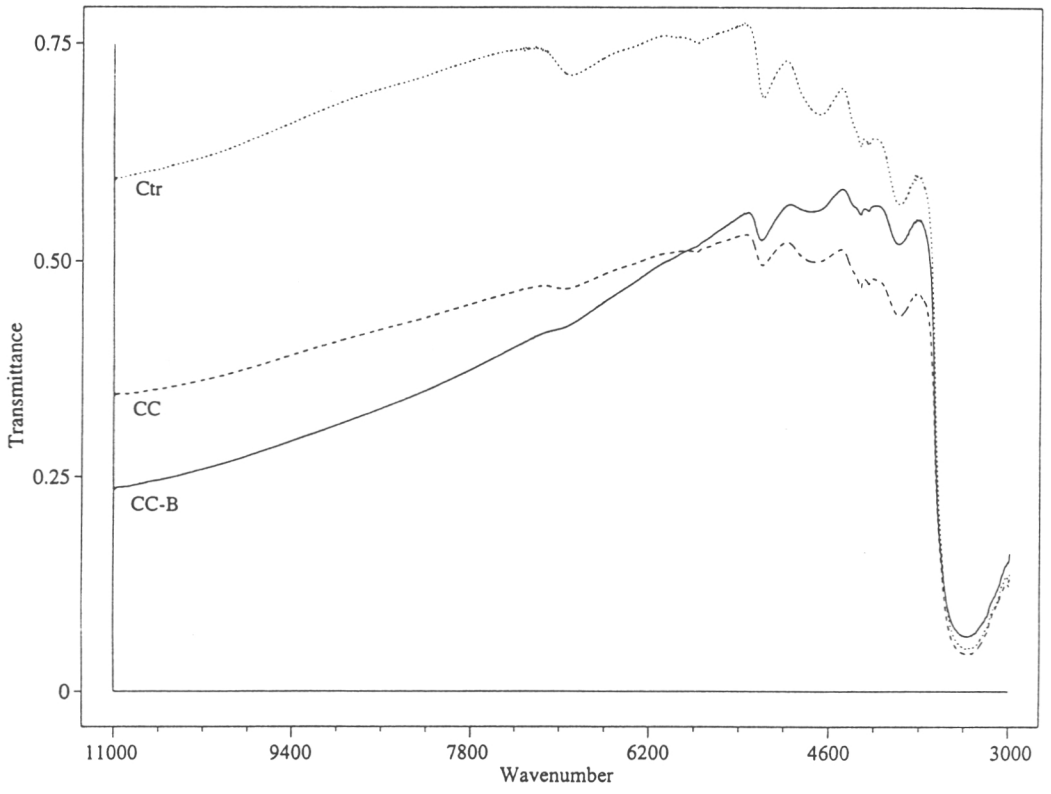

Fig. 1. Near infrared reflectance spectra of one representative humus sample from each of the treatments: control (Ctr), clear-cut (CC) and clear-cut + burned (CC-B).

CC-B humus samples, the amount of NH_4^+-N was initially significantly higher than Ctr, but in both cases it fell to the Ctr level during incubation. Although NH_4^+-N had disappeared in both samples, nitrification had occurred only in CC-B humus where the increase in NO_3^--N was from 13.9 to 85.7 $\mu g\, g^{-1}$ dry wt over 6 weeks. In CC humus, the change in nitrate–N was negative.

DISCUSSION

The reduction in C_{mic} following clear-cutting (CC) was detected by both variables measuring total microbial carbon (C_{mic}): fumigation–extraction (FE) and SIR methods. Values from both methods were correlated significantly, but SIR gave unexpectedly high values for C_{mic}. The FE method measures total C_{mic} from cells vulnerable to lysis by chloroform. The

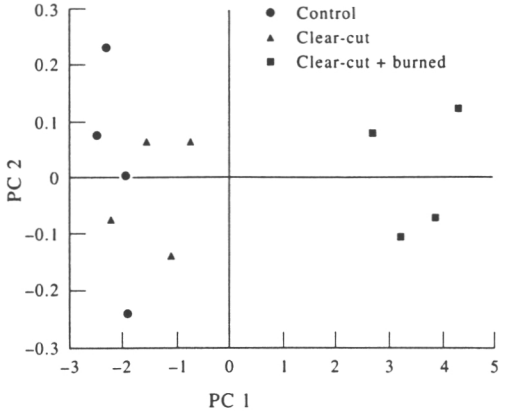

Fig. 2. A principal component analysis of the humus NIR spectral data.

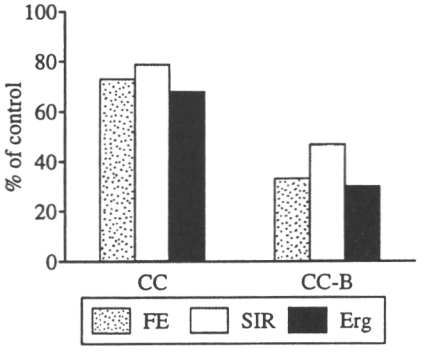

Fig. 3. Treatment-induced reduction of microbial biomass carbon measured by FE and SIR methods, and fungal ergosterol (Erg). See Fig. 1 for treatment symbols used.

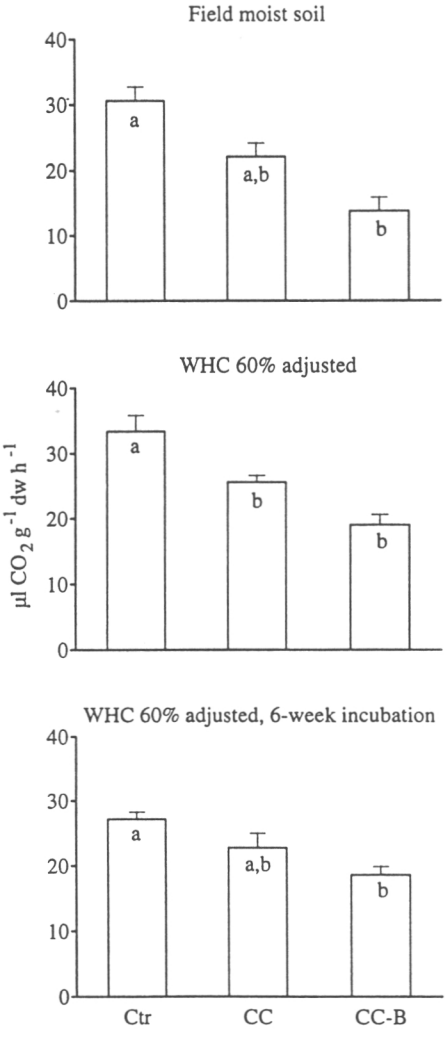

Fig. 4. Microbial respiration rate of field-moist and moisture-adjusted humus samples. Bars indexed by the same letter are not significantly different at the $P < 0.05$ level (Tukey's test). See Fig. 1 for treatment symbols used.

SIR method takes into account only aerobic, glucose-metabolizing microbes, and gave higher C_{mic} values than FE throughout the study. This is obviously a distortion caused by an unsuitable conversion equation used to convert the measured respiration rates to C_{mic}. The conversion factors published to date have been determined in soils with relatively low glucose-induced respiration rates and consequently low C_{mic}. Conversion factors have been obtained using glucose-induced respiration rates (in ml CO_2 100 g^{-1} soil dry wt h^{-1}) between 0.35 and 6.5 (Anderson and Domsch, 1978), 1.0–3.5 (Sparling *et al.*, 1990) and 0.15–4.9 (Kaiser *et al.*, 1992). These rates are, however, remarkably low compared to our measured values from 10.9 to 31 ml CO_2 100 g^{-1} h^{-1}. A reliable conversion factor for high biomass values does not yet exist. The regression equation we used to convert our primary results from FE to C_{mic} is determined in boreal ecosystems under comparable conditions and C_{mic} (Martikainen and Palojärvi, 1990). Thus, our results obtained by FE should be more reliable than those measured by SIR.

Table 2. The percentage of the variance of the biological variables explained by partial least square (PLS) regression using the first component of the spectral data from near infrared reflectance analysis

y-variables	Variance explained (%)
C_{mic}-FE	82.2
C_{mic}-SIR	74.8
Erg	79.3
Respiration	62.0

See Table 1 for symbols used. Respiration refers to field moist soil.

The reduction in humus ergosterol concentration was even steeper than that of the total C_{mic}. Ergosterol is a fungal membrane component and its amount has been shown to correlate with fungal surface area (West *et al.*, 1987). The reduction in fungal biomass after CC is in accordance with Bååth (1980) who showed that total fungal lengths measured by the agar-film method decreased after clear-cutting in Central Sweden and the reduction was consistent over three growing seasons following treatments. The number of active ectomycorrhizal roots have been shown to decrease sharply during the first year after clear-cutting, because the root system quickly loses its ability to support ectomycorrhizae after stem removal (Harvey *et al.*, 1980). The reduction in mycorrhizal fungi is probably reflected in the observed diminished concentration of ergosterol.

Another consequence of vegetation removal is the cessation of annual litter fall and root exudation. The fine roots of the felled trees become subject to degradation. The absence of covering vegetation results in wider variation in soil temperature and moisture. These events may have either positive or negative effects on microbial biomass. The absence of litter fall should be compensated for by the logging residue left on the forest floor and the decomposition of dead root material below ground. The initial effect of clear-cutting can thus be beneficial to microbes, especially the bacterial portion, as pointed out by Lundgren (1982), who reported an initial, 2 yr long increase in the number of bacteria after clear-cutting, and a subsequent fall below the control level. In our study, the time of sampling represented the fourth growing season after logging. The biomass may have peaked after logging, but it has fallen clearly below the level of a standing forest by the fourth summer. By that time, the bacterial biomass estimated as the bacterial phospholipid fatty acids concentration g^{-1} soil dry wt by the method described by Bååth *et al.* (1992) was 23% lower than in the control (Bååth, Frostegård, Pennanen and Fritze, unpublished

Table 3. Nitrogen transformations ($\mu g\ g^{-1}$ dry soil) during a 6 week incubation of control, clear-cut and fire treated soils

	Control		Clear-cut		Clear-cut + burned	
Variable	Mean	SE	Mean	SE	Mean	SE
NH_4^+-N						
0 weeks	46.7a	6.77	167b	25.8	145b	5.78
6 weeks	30.0	2.59	53.5	21.3	16.9	5.48
ΔN	−16.7a	5.35	−114b	20.4	−128b	9.70
NO_3^--N						
0 weeks	0.66a	0.033	1.13a	0.28	13.9b	2.72
6 weeks	0.54	0.012	0.98	0.21	85.7	20.9
ΔN	−0.12a	0.039	−0.16a	0.37	71.9b	23.3

Negative Δ N indicates consumption and positive Δ N formation of ammonium or nitrate nitrogen during the incubation. Means followed by the same letter are not significantly different at the $P < 0.05$ level (Tukey's test).

results). This reduction was less severe than the 32% reduction in fungal ergosterol (Fig. 3). Obviously bacteria suffer from CC less than the fungal population.

Prescribed burning of the clear-cut sites (CC-B) caused an additional, significant reduction in microbial biomass. The reduction in C_{mic} from the Ctr level caused by CC-B was 67 and 53% as measured by FE and SIR, respectively. The reduction caused by CC alone was respectively 27 and 21%. However, it has been shown that the reduction in C_{mic} after burning is not dependent on previous CC since prescribed burning in an uncut forest stand resulted in a 70% decline in C_{mic} (Fritze *et al.*, 1994b) which is close to the 67% decline from Ctr to CC-B observed in this study. This decline in microbial biomass after burning is either a direct effect of fire or a consequence of fire-induced modifications in the soil. Heat during fire has a sterilizing effect on soil (Bollen, 1969; Dunn *et al.*, 1985) but burned soil is always gradually recolonized from propagules surviving in soil (Theodorou and Bowen, 1982) or introduced from adjacent areas (Jalauddin, 1969). The changes in nutrient status after burning are similar to those observed after ash fertilization and they have not been shown to reduce C_{mic} (Fritze *et al.*, 1994b). Since the rise in humus pH is not responsible either for the decline in C_{mic} (Fritze *et al.*, 1994b), two fire-induced modifications in soil can be presented to explain the observed decrease in microbial biomass of the CC-B plots: (1) lower soil moisture content and (2) altered structure of soil organic matter (SOM).

At the moment of sampling, CC-B soil had the *lowest moisture content* (60% wet wt) compared to CC or Ctr (75 and 73% wet wt, respectively). The CC-B soil is probably the driest throughout the growing season due to a lower water holding capacity (WHC) and increased evaporation. This obviously limits microbial proliferation since both fungal and bacterial biomass have been shown to correlate positively with soil moisture (Söderström, 1979; Clarholm and Rosswall, 1980). Respiration of CC-B soil showed the strongest response to water addition (WHC 60%) which indicated water limitation. Incubation for 6 weeks without added substrate did not lower the respiration rate of CC-B soils to the same extent as in Ctr and CC soils. Obviously, CC-B soils did not suffer from substrate exhaustion even though the SOM content of these soils was lower than in Ctr and CC soils. A reduction in C_{mic} after burning is observed even if the humus moisture and amount of SOM remained unchanged, but a reduced moisture content results in a sharper decrease in C_{mic} (Pietikäinen and Fritze, 1993).

The reduced amount of C_{mic} independent of soil moisture can be a result of *altered humus quality*. Almendros *et al.* (1990) reported the existence of a 'pyromorphic humus'. They observed that fire caused structural modifications in humic and fulvic acids and changes in their colloidal properties. The fire-induced, i.e. 'pyromorphic' compounds may have some inhibitory effect on microorganisms. Widden and Parkinson (1975) observed extracts of burned soils to inhibit spore germination of *Penicillium* and *Trichoderma* species. Burning might effect C_{mic} via the chemically-modified 'pyromorphic SOM'. The quality of SOM is reflected in the near infrared reflectance (NIR) spectra (Figs 1 and 2), which indicate profound but as yet unidentified differences between treatments. Nilsson *et al.* (1992) and Palmborg and Nordgren (1993) presented a hypothesis that the NIR spectra of the SOM can be used in mathematical modelling of biological responses. They found that after NIR spectroscopical analysis of the forest humus, over 80% of the variation in soil basal respiration could be explained by NIR. In addition, Fritze *et al.* (1994a) reported that over 80% of the variation of C_{mic} could be explained by NIR. Their results are supported by our study, since a partial least square regression (PLS) constructed from the humus NIR variables and C_{mic} values explained over 80% of the variation in microbial biomass in the study area. It seems reasonable that the humus quality, as analysed by NIR, determines the amount of microbial biomass. To test this hypothesis, we used the PLS regression model, constructed from humus NIR and C_{mic}-FE variables from a wood ash fertilization and burning experiment (Fritze *et al.*, 1994a)

and predicted the C_{mic}-FE values of this study by using the respective NIR variables (see Materials and Methods). The predicted values (Table 4) are somewhat lower than the observed ones, but they sort the treatments into the observed, diminishing order Ctr > CC > CC-B. Thus, the humus NIR spectrum is a strong but not the only predictor of the amount of C_{mic}.

Prescribed burning severely affected soil fungi. The amount of fungal ergosterol fell by 56% compared to CC. In general, burning has been shown to favor bacterial populations over fungal (Bissett and Parkinson, 1980), which is consistent with the observed minor 33% reduction calculated on dry weight basis in the bacterial phospholipid fatty acids between CC and CC-B (Bååth, Frostegård, Pennanen and Fritze, unpubl. results). Entry *et al.* (1986) showed the proportion of bacteria in the soil microflora to increase from 20% of total biomass in control soil to 80% in burned soil. This increase in the proportion of bacterial biomass may be due to higher soil pH, better ability to survive during burning or better adaptation to utilization of pyromorphic compounds. Heating experiments have shown that bacteria are more tolerant to heat than fungi (Bollen, 1969; Dunn *et al.*, 1985). The disappearance of certain fungal populations may lead to lower decomposition capacity of a disturbed soil since fungi are responsible for the degradation of many recalcitrant substances.

A shift in bacterial and fungal proportions of C_{mic} in the burned humus is obvious, but, simultaneously, the species composition within both groups may change. The high specific respiration rate qCO_2 of CC-B can be an indication of pioneer species exhibiting high respiration. An elevated qCO_2 is often observed in soils with low C_{mic} and during initial succession, e.g. after prescribed fire (Fritze *et al.*, 1993) or during soil reclamation (Insam and Haselwandter, 1989).

Both CC and CC-B soils were supplied with equal amounts of ammonium, but only in the fire-treated soils was there oxidation of ammonium to nitrate. Lack of ammonium has not been the limiting factor for nitrification in CC soil compared, e.g. to the postfire situation on barren land, where NH_4^+-N concentration rose only to 26 $\mu g\, g^{-1}$ dry wt and consequently, no nitrification was detected (Fritze *et al.*, 1994b). Factors other than substrate depletion responsible for limiting nitrification could be low pH, existence of chemical inhibitors or competition with heterotrophs. Our results support the hypothesis of Bauhus *et al.* (1993) who claimed that soil heating reduces competition between autotrophs and heterotrophs and that ash-derived nutrients, such as Ca and K, stimulate nitrification. Martikainen (1984) has shown that ash fertilization and the resulting higher pH increased nitrification. The nitrate concentration of the CC-B soils was higher than in the other treatments in both the fresh (0 weeks) as well as in the incubated (6 weeks, Table 3) samples which implies that nitrate formation actually occurs in the field, not only during laboratory incubations. Under optimal conditions, the nitrate formed is immobilized in the pioneer vegetation, and is only gradually liberated, which prevents possible nitrate leaching.

Table 4. Predicted* microbial biomass C (C_{mic}-FE; $\mu g\, g^{-1}$ dry wt) from the NIR variables. Standard error is presented

Treatment	Measured C_{mic}	Predicted C_{mic}
Control	7281 ± 439	5438 ± 186
Clear-cut	5293 ± 222	4464 ± 389
Clear-cut + burned	2398 ± 218	1548 ± 94

*Predicted with the partial least square (PLS) regression model constructed from humus NIR (near infrared reflectance) variables and C_{mic}-FE values from Fritze *et al.* (1994a).

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Paper IV

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IV

Soil Biology & Biochemistry (in press)

DOES SHORT-TERM HEATING OF FOREST HUMUS CHANGE ITS PROPERTIES AS A SUBSTRATE FOR MICROBES?

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Short title: Microbial community structure in heated humus

Summary—Prescribed burning is known to reduce the size of the microbial biomass in soil, which is not explained by preceding clear-cutting or the effects of ash deposition. Instead, burning induces an instant heat shock in the soil, which may either directly kill soil microbes or indirectly alter the soil organic matter. We heated dry forest humus at temperatures from 45°C to 230°C, inoculated them to ensure equal opportunities for microbial proliferation, and incubated the heated humus samples at 14°C. After one, two, four and six months we studied the microbial community structure of the samples by determining the phospholipid fatty acid pattern (PLFA), microbial substrate utilization pattern using Biolog Ecoplates, and total microbial biomass (C_{mic}) by substrate-induced respiration (SIR). The chemical structure of humus was scanned by FTIR and ^{13}C-NMR spectroscopy. Heating at 230°C caused changes in the chemical structure of the humus as indicated by FTIR spectroscopy, increased the pH of the humus by 1.1 units, reduced C_{mic} by 70% compared with the control and caused changes in substrate utilization patterns and proportions of PLFAs. More interestingly, the heat treatments from 45°C to 160°C, which did not increase humus pH, resulted in differences in both microbial community structure and substrate utilization patterns. The severely heated samples (120-160°C) were relatively richer in 16:1ω7t, cy19:0 and 18:1ω7, while the mildly heated samples (45-100°C) showed higher proportions of 16:1ω5, 16:1ω9, 10me16:0 and a15:0. The *t/c* ratio calculated from *trans* and *cis* configurations of 16:1ω7 increased from one to six months in the severely heated humus, possibly indicating nutrient deprivation. The control showed a decreasing *t/c* ratio and a stable amount of C_{mic} indicating sufficient amount of decomposable organic matter. After incubation of one month, similar amounts of C_{mic} had re-established in 160°C-treated and control samples. However, the C_{mic} in 160°C-treated samples decreased over five months. This might have been caused by a heat-induced flush of easily decomposable carbon, which was later exhausted. We conclude that changes in chemical properties of humus during dry heating at 230°C were capable of causing changes in microbial community structure of the humus.

INTRODUCTION

Prescribed burning causes a profound reduction in soil microbial biomass, which may last up to ten years (Fritze *et al.*, 1993; Prieto-Fernández *et al.*, 1998). Prescribed burning as a forestry practice in the northern boreal forests is preceded by clear-cutting, which may also lower the size of the soil microbial biomass (Bååth, 1980; Lundgren, 1982). However, the overall decline in soil microbial biomass caused by clear-cutting accounts for less than half of that caused by prescribed burning (Pietikäinen and Fritze, 1995). Thus, it is concluded that the reduction in soil microbial biomass after prescribed burning is mainly a consequence of fire.

Firing of vegetation and litter causes a dual effect on soil. It induces a transient heat shock in the upper soil horizon and produces a layer of nutrient rich ash on the forest floor, both phenomena being capable of affecting microbial biomass in soil. Ash fertilization has been shown to either slightly increase (Frostegård *et al.*, 1993a) or decrease (Bååth *et al.*, 1995) microbial biomass measured as the total amount of microbial phospholipid fatty acids in soil. The effect of ash fertilization is mediated mainly via its capacity to raise soil pH, and thus it resembles the effect caused by liming. Both procedures have a stimulatory effect on soil microflora, as shown by an increase in the proportion of culturable bacteria and specific respiration rate (Bååth and Arnebrant, 1994). In order to exclude the drastic pH effect resulting from the combustion of organic matter and formation of basic cations in ash, we focused on the changes caused solely by an instant heat shock in the soil.

The uppermost layer of soil is confronted with the most severe effects of burning. Finnish forest soils have a thick organic layer (L+F+H), which is attacked most severely by fire. The maximum surface temperatures during prescribed burning may be up to 800°C (Vasander and Lindholm, 1985), but the damp humus of the organic layer is an excellent thermal insulator, which protects the underlying soil from extreme temperatures (Uggla, 1958). Because the temperature of the humus cannot reach values above 100°C before all water has evaporated, a sweating zone is formed under the burning zone. This sweating zone regulates the temperature in the underlying soil. Thus, the determination of the exact effects of soil heating during prescribed burning in the field is somewhat obscure, because, on the one hand, fire severity is patchy and determined by the distribution of burning fuel, and, on the other hand, the moisture of the humus determines the depth of the sweating zone and thus the extent of the humus affected. To overcome these difficulties we studied the effect of temperature on humus in a laboratory experiment, where humus was heated to temperatures ranging from 45 to 230°C. A thin layer of dry humus was used in the experiment in order to prevent the formation of a steep temperature gradient and a sweating zone.

During experimental soil heating, changes in both the quality of soil organic matter (Knicker *et al.*, 1996; Fernández *et al.*, 1997) and the biomass and activity of soil microbes have been reported (Diaz-Raviña *et al.*, 1992, 1996; Grasso *et al.*, 1996). The aim of our work was to study the chemical changes in humus caused by dry heating, and follow the microbial succession in the heated and inoculated samples during laboratory incubation for six months. We detected the possible changes caused by heat in the humus by FTIR and NMR spectroscopy and evaluated the capacity of the heated humus to act as a substrate for the restoration of the

microflora. We followed the succession of the post-treatment microbial communities using the phospholipid fatty acid (PLFA) method and the microbial substrate utilization pattern with Biolog Ecoplates.

MATERIAL AND METHODS

Soil sampling and the heating procedure

Approximately 100 dm^3 fresh forest humus was collected for the laboratory experiment in a mixed stand of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) near the field station of the Finnish Forest Research Institute in Ruotsinkylä, Tuusula in southern Finland (60°20'N, 25°E). After removing the moss layer and the dwarf shrub vegetation, which consisted mainly of *Vaccinium myrtillus* L., the forest humus layer (F+H) was removed in large slabs, placed in plastic boxes and transported to the laboratory. The soil was passed through a 2.8 mm sieve before air-drying at 45°C. Before air-drying a subsample of the fresh humus was taken for characterization of its microbiological properties and preparation of the inoculum. The organic matter (o.m.) percentage and pH of the humus were 75.4% (SEM 0.17), and 3.85 (SEM 0.013), respectively. The fresh humus contained 7.13 mg microbial C g^{-1} o.m. (SEM 0.031) and the ratio of fungal to bacterial phospholipid fatty acids was 0.37 (SEM 0.017). For description of methods see below.

The sieved and air-dried humus was thoroughly mixed and divided into 128 portions, each having a mass of 25 g. The air-dried humus contained ca. 4% water. One subset of the samples (n=16) was left untreated. This set experienced only one temperature (45°C) during air-drying, and will be referred to as the control. The remaining seven sets of samples (7 x 16 = 112) were subjected to seven different temperature treatments: 60°C, 80°C, 100°C, 120°C, 140°C, 160°C and 230°C. In the heating procedure, each 25-g portion of humus was spread on a separate metal tray (10 cm x 20 cm) in a 2-cm layer. During heating the actual temperature inside the humus was continuously recorded with a K-type thermocouple, and the treatment was considered to have begun only when the internal temperature of the humus had reached the specified value. The temperature was maintained at this level for 10 minutes before the samples were allowed to cool. The heating procedure differed only for the set of samples treated at 230°C, because in the presence of oxygen dry humus material starts to smoulder at about 180°C. These samples were heated in a muffle furnace in glass vessels where the limited availability of oxygen reduced smouldering and charcoal formation. The amount of organic matter as loss-on-ignition and the carbon-to-nitrogen ratio were measured after the heat treatments to estimate the amount of organic matter combusted during heating.

The treated samples were placed in large glass vessels (volume 0.3 dm^3), and the dry samples were gradually moistened to a level of 50% of the water holding capacity (WHC). The WHC was determined separately for each subset of samples, but since it did not differ among samples treated at 45-160°C (average WHC: 6.4 ml g^{-1} dry matter), a common value for these samples was used. This value was somewhat lower than the value for fresh humus (7.0 ml g^{-1} d.m.), which is obviously a consequence of air-drying; when rewetted, soils do not absorb the same amount of

water they contained originally. The samples heated at 230°C became water repellent, and thus the WHC was only 3.5 ml g^{-1} d.m. An inoculum was added to provide each humus sample with the original microflora. For the preparation of the inoculum, 60 g fresh humus was mixed with 1200 ml purified water, and the suspension was filtered through glass wool. An inoculum of 5 ml was added to every humus sample as a part of the moisture adjustment. The inoculated and moistened samples were incubated at 14°C in the dark, placed in a random order in the incubator. The moisture of the humus was controlled and the sample mixed every two weeks. Four samples per temperature treatment were drawn aside from the experiment after 1, 2, 4 and 6 months.

Soil analyses

The Fourier-transform infrared (FTIR) spectra of the mortared samples (all treatments: 45-230°C) were measured with a Perkin Elmer System 2000 FTIR instrument using the KBr disk technique. Samples were scanned 32 times with a resolution of 4 cm^{-1} using a scan range from 4000 to 370 cm^{-1}. A cross-polarization magic-angle (CP/MAS) technique, which allows acquisition of ^{13}C-NMR spectra from solid samples, was used, to replenish the information from the IR-spectra. Four samples (45°C, 80°C, 120°C and 160°C) were chosen for the NMR analysis. The spectra were obtained on a Bruker AC 300 P spectrometer operating at 75.5 MHz. The following instrumental conditions were used: contact time 2 ms, repetition delay 2.5 s, spectral width 41.7 kHz, exponential window function 50 Hz, ^{13}C 90° (Hartmann-Hahn) pulse 5.3 µs, number of data points 16 k and number of transients 3000. Cross-polarization magic angle spinning at 6 kHz was utilized.

Microbial biomass carbon (C_{mic}) was determined from a humus subsample equal to 2 g d.m. using the substrate-induced respiration (SIR) method (Anderson and Domsch, 1978). The subsamples were moistened with a glucose solution and water to optimum moisture (60% of WHC) and a glucose concentration of 20 mg ml^{-1} soil water. The carbon dioxide which evolved was measured after two hours and calculated as mg C_{mic} g^{-1} o.m. (Priha and Smolander, 1994).

Humus pH was measured in humus-water suspension (3:5 v/v). Humus dry matter was determined after drying the subsamples overnight at 105°C. The amount of organic matter was determined as loss-on-ignition by heating at 550°C for 4 h and weighing the remaining mineral material. Total carbon and nitrogen were determined by dry combustion with a Leco CHN-1000 analyzer.

The phopholipid fatty acids were extracted from moist humus subsamples of 0.5 g (all samplings, n = 128) by the method described by Frostegård *et al.* (1993b). A total number of 34 fatty acids was identified. For fatty acid nomenclature see e.g. Frostegård *et al.* (1993b). Twelve individual fatty acids according to Frostegård and Bååth (1996) were regarded as having bacterial origin and 18:2ω6,9 was taken to represent fungal biomass. The total amount of phospholipid fatty acids (total PLFA) was calculated from nonadecanoate (19:0) as an internal standard and expressed as µmol g^{-1} o.m. In addition to C_{mic}, the value for total PLFAs was used as an estimate of microbial biomass, since PLFAs occur in the membranes of all living organisms, and they are not storage compounds (Frostegård *et al.*, 1991).

The substrate utilization pattern was analyzed using Biolog Ecoplates, which

contain 31 different substrates + water (Biolog, Inc., Hayward, California, USA). Subsamples for the Biolog analysis were taken at two samplings: after incubation of one and six months. Subsamples of 2.5 g were stored frozen before the analysis. This might have affected the substrate utilization patterns of the samples, since not all microbes survive freezing. However, all the samples were treated identically. For the inoculation of the Ecoplates the subsamples were melted and 50 ml purified water was added. The humus-water suspension was shaken (250 rev min^{-1}) at 4°C for 1 h to gently release bacteria from the humus to the aqueous phase. The suspension was centrifuged (10 min, 750 x *g*) and the supernatant filtered through quartz wool. The filtrate was diluted 1:10 and the wells of the Ecoplate were filled with 150 µl. After inoculation the plates were incubated at 20°C and 35% relative humidity for seven days. The absorbances of the plates were recorded once a day with a Labsystems Multiscan MS plate reader using a 590-nm filter. The actual reading times differed for the two sets of samplings (1 and 6 months) but the last reading time for both sets was 168 h.

Data analyses

All the results of the microbial biomass are expressed per humus organic matter content to reduce the effect of carbon combusted from the samples heated at the highest temperatures. The differences in percentages of individual PLFAs and scores of the first principal component (PC1) between temperature treatments were detected separately for each sampling date using one-way ANOVA. Because of unequal variation within temperature treatments Kruskall-Wallis one-way ANOVA was used for testing the pH data. Differences in the amount of C_{mic} were analyzed by two-way ANOVA with treatment temperature and incubation time as grouping variables. Tukey's test was used for pairwise comparisons. The individual PLFAs were expressed as mole percentage (mol%) of the total PLFA content of the sample. The mol% values were subjected to principal component analysis (PCA) using the correlation matrix. All four samplings were analyzed separately in the PCA. The humus samples heated at 230°C were excluded from the PCA because of a limited number of detected PLFAs. The changes in the proportions of PLFAs in the 230°C-treated samples were detected by forming ratios of the detected PLFAs (n=11) to hexadecanoate (16:0), which is the most abundant PLFA of microbes. The ratios were calculated for the whole temperature range of the last sampling date (six months). For the Biolog data individual substrates were corrected for background absorbance by subtracting the absorbance of the control (water) well. The blank corrected absorbance values of the subsequent readings were used to calculate the total area under the absorbance curve. The calculation was made according to Sharma *et al.* (1997), except that the total area was not approximated per hour since the time of the last reading was 168 h for all the plates. The calculated values (area under the curve) were summed for each humus sample and the proportions of individual substrates from this total area were expressed as area%. The area percentage value is a measure of proportional substrate utilization efficiency, and is not affected by inoculum density. Before subjecting the area percentages to canonical discriminant analysis (CDA) the treatments were combined to form three groups: 1) mild heat 45-100°C, 2) severe heat 120-160°C and 3) extreme heat

230°C. The CDA was used to find out how well the three differently heated groups (1-3) could be separated given the values for the substrate utilization efficiency.

RESULTS

The visual appearance of the humus did not change at 60-160°C when compared to the control (45°C, air-dried). The percentage of organic matter (o.m.) and carbon-to-nitrogen ratio (C:N) did not differ between samples heated at 45-160°C. The values for o.m., C:N ratio and pH with significant differences between temperature treatments are shown in Table 1. Heating at 230°C with limited oxygen supply gave the humus a black, glossy appearance, and it contained small charcoal particles. The percentage of o.m. and C:N ratio in the 230°C-treated sample were significantly lower than in control (Table 1). Air-drying and rewetting raised the pH value of the humus from the level in the fresh humus (3.85) to ca. 4.3. Heating at 160°C lowered the pH value by 0.6 units compared to control, but the difference in pH between 160°C-treated and control samples decreased during incubation, being less than 0.2 units at six months. The difference in pH was not significant between samples heated at 45-160°C (Table 1). Heating at 230°C was capable of raising pH to 5.4.

The FTIR spectra of three samples (45°C, 160°C and 230°C) are presented in Fig. 1a. The spectra show the following absorbances: broad band in the 3380-3340 cm^{-1} region, H-bonded OH stretch, NH stretch; sharp peaks at 2920 cm^{-1} and 2850 cm^{-1}, aliphatic C-H stretch; shoulder at 1720 cm^{-1}, C=O stretch; broad band around 1640 cm^{-1} (the bands between 1660 and 1600 cm^{-1} are caused by several overlapping functional groups; different kind of C=C including conjugation with C=O, unconjugated C=O, C=N, COO^- and N-H absorptions), sharp peak at 1515 cm^{-1}, aromatic skeletal vibrations; broad overlapping band system from 900 to 1200 cm^{-1} with maximum around 1040 cm^{-1}, C-O stretch which is typically attributed to the carbohydrates; however, this area is very complicated and a definite interpretation cannot be given. The spectral interpretation is based on data by Celi *et al.* (1997), Holmgren and Nordén (1988), Lin-Vien *et al.* (1991) and Stevenson and Goh (1971).

The 230°C-treated humus gave a quite different spectrum compared to the control. The frequency of the OH/NH band at 3380 cm^{-1} was shifted to 3350 cm^{-1}. This shift may indicate decomposition of compounds containing NH groups. Respectively, the C=O frequency was shifted about 10 cm^{-1} lower. Changes in the 1660-1600 cm^{-1} region can be attributed to a decreased amount of amines and amides and on the other hand, increased amount of aromatic compounds. The frequency shift at the carbohydrate region (1030-1080 cm^{-1}) is also an evidence of an increased aromatic character of the 230°C-treated sample. All the samples treated at lower temperatures (45-160°C) gave similar spectra.

The NMR technique was used to reveal possible differences in the structure of the 45°C, 80°C, 120°C and 160°C-treated samples. A typical spectrum is shown in Fig. 1b. The spectrum was divided into seven regions representing distinct groups of carbon signals: 1) aliphatic carbons, 2) carbon bonded to O and N heteroatoms, including carbohydrates, 3) olefinic carbons C=C, 4) olefinic and aromatic carbons,

Table 1. *Mean values of organic matter (percentage of d.m.) as loss-on-ignition, carbon-to-nitrogen ratio (C:N) and pH of the heated humus samples (±SEM, n=4). The values marked with the same letter within a row are not significantly different at P < 0.05 (one-way ANOVA followed by Tukey's test for loss-on-ignition and C:N, Kruskal-Wallis one-way ANOVA for pH data).*

Variable	Incubation time	Treatment temperature							
		45°C	60°C	80°C	100°C	120°C	140°C	160°C	230°C
Loss-on- ignition (%)	prior to incubation	76.0^{a} ±1.5	74.5^{a} ±1.0	74.4^{a} ±0.5	74.5^{a} ±0.8	74.5^{a} ±0.5	73.5^{a} ±0.8	72.4^{a} ±0.8	65.8^{b} ±0.5
C:N	prior to incubation	27.2^{a} ±0.3	27.1^{a} ±0.3	28.0^{a} ±0.3	27.7^{a} ±0.2	27.3^{a} ±0.3	28.1^{a} ±0.2	27.9^{a} ±0.2	24.8^{b} ±0.1
pH	1 month	4.30^{ab} ± 0.33	4.30^{ab} ± 0	3.83^{ab} ± 0.28	4.30^{ab} ± 0.07	4.30^{ab} ± 0.14	4.10^{ab} ± 0.04	3.83^{a} ± 0.11	5.40^{b} ± 0.12
	2 months	4.38^{ab} ± 0.03	4.35^{ab} ± 0.04	4.43^{ab} ± 0.01	4.43^{ab} ± 0.03	4.36^{ab} ± 0.06	4.24^{a} ± 0.02	4.19^{a} ± 0.03	5.04^{b} ± 0.13
	4 months	4.53^{abc} ± 0.01	4.51^{ab} ± 0.01	4.55^{abc} ± 0.01	4.58^{bc} ± 0.01	4.57^{abc} ± 0.01	4.51^{abc} ± 0.04	4.37^{a} ± 0.03	4.89^{c} ± 0.10
	6 months	4.59^{ab} ± 0.01	4.59^{ab} ± 0.02	4.61^{ab} ± 0.03	4.64^{ab} ± 0.03	4.63^{ab} ± 0.03	4.58^{ab} ± 0.03	4.44^{a} ± 0.01	5.22^{b} ± 0.14

Table 2. *Signal intensities in ^{13}C-NMR spectra of the humus samples.*

Region*	Chemical shift (ppm) range	Treatment temperature			
		45°C	80°C	120°C	160°C
1	0 - 38	3.2	3.1	2.7	3.6
2	38 - 85	50.3	50.1	50.3	44.5
3	85 - 104	12.2	12.6	12.4	11.5
4	104 - 131	12.3	12.7	13.2	12.3
5	131 - 150	7.4	7.2	7.2	7.0
6	150 - 184	10.1	10.1	10.1	10.1
7	184 - 225	3.2	3.1	2.7	3.6

* *Regions as determined by Malcolm (1990) and Barančíková et al. (1997). For a detailed description of the regions see Results section.*

5) aromatic quaternary carbons and oxygen or nitrogen bonded aromatic carbons, 6) carboxylic carbons and amide carbonyl carbons, 7) aldehyde and ketone carbonyl carbons (Malcolm, 1990; Barančíková *et al.*, 1997). Regions were integrated separately and valleys between broad resonances were used as integration cut-off points. As indicated by Table 2, the spectra of the four samples did not differ in any region.

Microbial biomass

The amount of microbial biomass restored in the air-dried control humus one month after the inoculation was 44% of the value in the native, fresh humus. The amount of C_{mic} in the control fluctuated slightly during the six-month incubation: first it decreased for four months after inoculation, but then recovered (the left column in Fig. 2). Six months after the inoculation the C_{mic} was 14% higher than at one month sampling. This rather stable level of microbial biomass in the air-dried samples proved that the conditions during incubation were not harmful to microbial life. Total PLFAs, which can also be used as a measure for microbial biomass, followed a pattern similar to that of C_{mic}. The correlation between these variables was 0.63 (P<0.001, n=128).

The long incubation period hindered only the growth of fungi, which was indicated by a very low ratio of fungal to bacterial phospholipid fatty acids. In all the samples treated at 45-160°C, the fungal/bacterial PLFA ratio remained at ca. 0.1 during the incubation, while the value in the native, fresh humus was 0.37. This value was typical of the humus horizon of the boreal forests (Frostegård and Bååth, 1996).

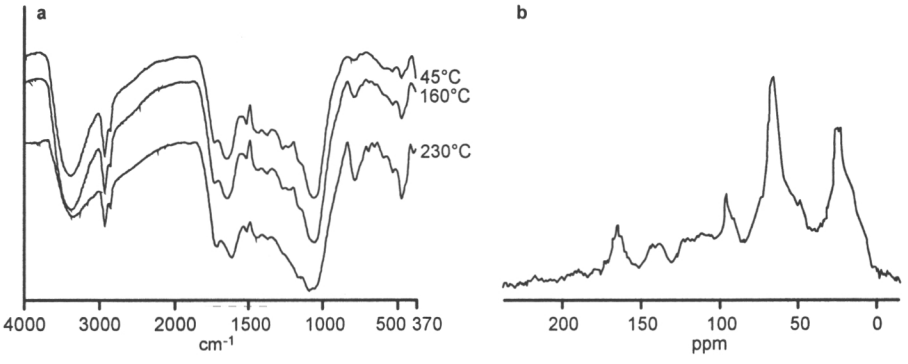

Fig. 1. (a) FTIR spectra of three heated humus samples; control (45°C), 160°C and 230°C-treated. Since the treatments 45-160°C gave similar spectra, only the highest and lowest temperature treatments are shown. (b) Representative solid state CP/MAS ^{13}C-NMR spectrum of the 160°C-treated humus sample. Other spectra are not shown, since they did not differ between treatments.

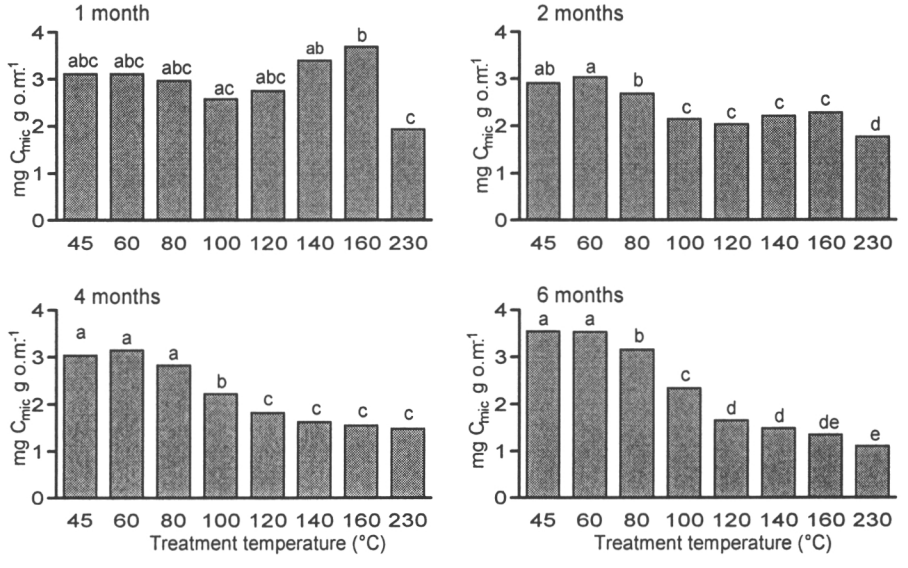

Fig. 2. Microbial biomass carbon (C_{mic}) as measured by substrate-induced respiration in humus heated at temperatures from 45 to 230°C after incubation for one, two, four and six months. The columns indexed by the same letter within a sampling are not significantly different at $P < 0.05$ level (one-way ANOVA followed by Tukey's test).

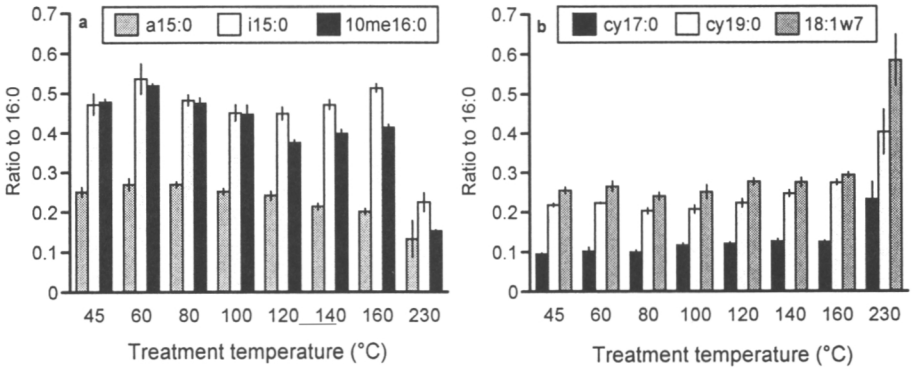

Fig. 3. Ratio of a15:0, i15:0, 10me16:0, cy17:0, cy19:0 and 18:1ω7 to the most abundant PLFA 16:0. These PLFAs showed the greatest changes in the subset detected consistently in the 230°C-treated samples. The ratios were calculated for the last sampling date (six months) using the whole temperature range 45-230°C. PLFAs decreasing in proportion to 16:0 are presented in (a), and those increasing are presented in (b). Error bars represent SEM.

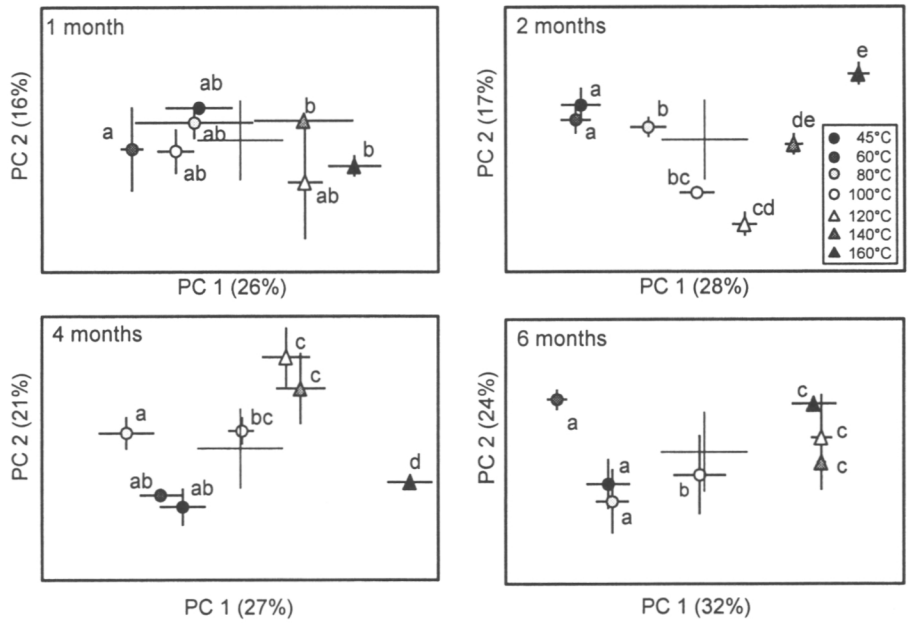

Fig. 4. PLFA patterns from humus heated at temperatures from 45°C to 160°C were subjected to principal component analysis. Each sampling (1, 2, 4 and 6 months) was treated separately in the analysis. The variance explained by each axis is presented as percentage, the cross represents the origin. The mean value for each treatment is presented, the bars indicate SEM. The labels indexed by the same letter within a sampling are not significantly different at $P < 0.05$ level (one-way ANOVA followed by Tukey's test).

Table 3. *Results of two-way ANOVA with microbial biomass carbon (mg C_{mic} g^{-1} o.m.) as dependent variable.*

Source of variation	df	SS	MS	F	*P*
Treatment temperature	7	$3.8 \cdot 10^7$	$5.4 \cdot 10^6$	189.5	< 0.001
Incubation time	3	$1.1 \cdot 10^7$	$3.6 \cdot 10^6$	125.7	< 0.001
Treatment temperature * Incubation time	21	$1.9 \cdot 10^7$	$8.9 \cdot 10^5$	31.4	< 0.001
Error	96	$2.7 \cdot 10^6$	$2.8 \cdot 10^4$		

The effects of heating on C_{mic} were highly time dependent (Table 3). Thus, the C_{mic} values were compared separately for every sampling date using one-way ANOVA. After one-month incubation the response of C_{mic} to soil heating followed a sigmoidal curve, with the highest C_{mic} values in the 160°C-treated samples (3.69 mg C_{mic} g^{-1} o.m.) and the lowest C_{mic} in samples treated at 100°C and 230°C (2.57 and 1.93 mg C_{mic} g^{-1} o.m., respectively). This curve started to flatten after two months and, at six months, the peak in the 160°C-treated humus had disappeared. At this time the microbial biomass decreased with increasing heating temperature (Fig. 2). No significant differences were observed between the 60°C-treated sample and control at any sampling time. On the other hand, the treatments at higher temperatures resulted in reduced C_{mic}, as observed at 2, 4 and 6 months. The lowest observed value for C_{mic} was 1.1 mg g^{-1} o.m. in the 230°C-treated samples after six-month incubation (ca. 30% of respective control).

Phospholipid fatty acid pattern

From the total number of 34 PLFAs detected in samples treated at 45-160°C, only 12 PLFAs were consistently found in the 230°C-treated samples. Since the proportion of individual PLFAs is expressed as a percentage of the total amount of PLFAs, the percentages for the 230°C-treated samples were not comparable with the rest of the samples, which had a complete PLFA chromatogram. Because of this limitation the 230°C-treated samples were not included in the multivariate analyses of the PLFAs. Instead, we present the ratio of individual PLFAs to hexadecanoic acid (16:0) for all treatments in the six-month sampling. In the 230°C-treated samples the ratios of a15:0, i15:0 and 10me16:0 to 16:0 decreased, while the ratios of cy17:0, cy19:0, 18:1ω7, 18:2ω6,9 and 20:0 to 16:0 increased. The ratios of 14:0, 16:1ω7c and 18:1ω9 to 16:0 did not change compared with the control. Fig. 3 presents the ratio to 16:0 for six individual PLFAs which showed the greatest changes.

In the humus samples heated at 45-160°C and supplied with the same inoculum, different types of microbial communities were established, which is illustrated by the separation of the samples in the principal components analysis (Fig. 4). When the

Table 4. *First principal component for sample sets incubated for 1, 2, 4 or 6 months. The loadings for the ten most highly weighted PLFAs are shown.*

Incubation time	1 month	2 months	4 months	6 months
% variance explained	26	28	27	32
PLFAs correlating positively with PC1, abundant in heated humus				
16:1ω7t		0.301	0.272	0.242
cy19:0		0.298	0.290	0.216
17:0		0.213	0.270	0.253
18:1ω7		0.282	0.309	
19:1a			0.226	0.229
cy17:0			0.216	0.220
i15:0	0.284			
15:0	0.283			
17:1ω8		0.248		
i14:0	0.251			
14:0	0.248			
16:1ω7c	0.184			
PLFAs correlating negatively with PC1, abundant in the control				
16:1ω5		-0.274	-0.253	-0.279
16:1ω9		-0.237	-0.240	-0.244
10me16:0		-0.260		-0.267
a15:0		-0.246	-0.257	
18:1	-0.262	-0.221		
10me18:0	-0.288			
i16:0				-0.264
10me17:0	-0.276			
18:0	-0.256			
a17:0	-0.195			
20:5			-0.212	
20:4				-0.211

Table 5. *Discriminant variables CAN 1 and CAN 2 for the sample set incubated for six months. The top ten carbon sources with the highest absolute loadings are shown.*

Discriminant variable	CAN 1		CAN 2
Eigenvalue	17.4		7.0
Positively weighted carbon sources			
i-erythritol	0.684	γ-hydroxy butyric acid	0.629
D-xylose	0.640	D-galacturonic acid	0.358
glycogen	0.625	glycyl-L-glutamic acid	0.318
D-cellobiose	0.560	D-mannitol	0.275
tween 40	0.389	cellobiose	0.258
		D-xylose	0.226
		glycogen	0.206
Negatively weighted carbon sources			
L-threonine	-0.593	pyruvic acid methyl ester	-0.358
D-glucosaminic acid	-0.555	L-phenylalanine	-0.356
L-asparagine	-0.459	L-arginine	-0.285
phenylethylamine	-0.421		
D-galactonic acid γ-lactone	-0.415		

scores of the first two principal components were plotted, it was clearly seen that samples heated at 160°C had high loading on PC1, and were clustered together. The control samples were always clustered with 60°C and 80°C-treated samples, and were negatively weighted along PC1, opposite to the samples from higher temperature treatments. At one-month sampling, the clusters still overlapped, but a temperature dependent trend was observed as the scores of the 140°C and 160°C-treated samples on PC1 were significantly different from the 60°C-treated sample (P=0.003, ANOVA). The significant differences between temperature treatments separately for each sampling (1, 2, 4 and 6 months) are shown in Fig. 4.

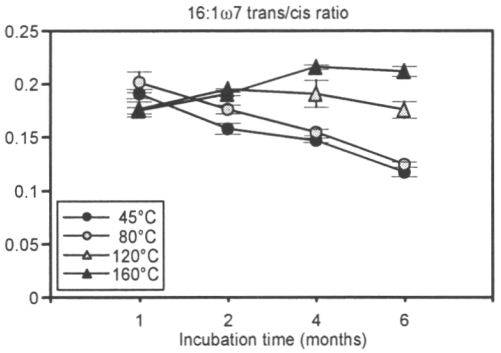

Fig. 5. Ratio of the configuration (trans/cis) of the double bond in 16:1ω7 was used to estimate the degree of environmental stress. For the sake of clarity, calculations for only four temperature treatments are presented. Error bars represent SEM.

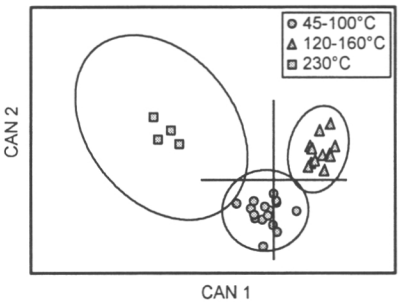

Fig. 6. Differences in substrate utilization patterns of microbial communities in humus subjected to varying heat treatments detected by canonical discriminant analysis. The treatment temperatures were combined to form three groups: mild heat (45-100°C), severe heat (120-160°C) and extreme heat (230°C). Results from incubation time of six months are presented. The confidence ellipses for predicted values at 95% level are drawn around each group.

The loading value of a variable (PLFA) expresses the strength of that variable in influencing the principal component in question. At one-month, a different subset of PLFAs contributed to PC1 as compared with the other sampling dates (Table 4). At two, four and six months, the same set of PLFAs had high loadings on PC1 and were responsible for the separation of the high and low temperature samples along PC1 (Fig. 4). When the PC1 scores were analyzed by ANOVA, there were significant differences between temperature treatments at each sampling. From two to six months, there were six PLFAs (16:1ω7t, cy19:0, 17:0, 18:1ω7, 19:1a and cy17:0) which had large positive loadings on PC1 and were found in relatively large proportions in the severely heated samples. They ranked in different order depending on the incubation time. The percentages of 16:1ω7t, cy19:0 and 18:1ω7 increased from 0.71, 3.65 and 4.26% in the control to 1.32, 4.70 and 5.02% in 160°C-treated humus, respectively ($P<0.005$). The PLFAs 17:0 and 19:1a followed the same pattern, but the increase as percentage units was negligible (< 0.2% units). cy17:0 also increased due to heating of humus by 0.5 percentage units ($P<0.001$), but reached its maximum values in 100°C or 120°C-treated samples. The PLFAs 16:1ω5, 16:1ω9, 10me16:0, a15:0 and 18:1 showed large negative loadings on PC1 and were dominant in the mildly heated samples (Table 4). The PLFA most clearly affected by heating was 16:1ω5. Throughout the incubation period the amount of

this PLFA was most abundant in the control (2.17 mol% at six months), and in heated humus it noticeably decreased (e.g. in 160°C-treated humus at six months 1.19 mol%, $P<0.001$).

The mole percentage values for PLFAs 16:1ω7t and 16:1ω7c were used to calculate the ratio of the *trans* and *cis* configurations of 16:1ω7 (i.e. the *t/c* ratio). During incubation the percentage of 16:1ω7t increased in severely heated (120-160°C) humus, while that of 16:1ω7c remained rather stable, which led to an increased *t/c* ratio (Fig. 5). In the mildly heated samples (45-100°C) the *t/c* ratio decreased gradually during incubation. At six months the values for control (0.12) and 160°C-treated humus (0.21) differed significantly ($P<0.001$).

Substrate utilization pattern

The substrate utilization patterns determined using the Biolog Ecoplates were assessed at one month and six month samplings. The clustering of the treatment groups at both samplings was similar in the discriminant analysis, so only the results for the six-month sampling are presented (Fig. 6). The first canonical discriminant variable (CAN 1) separated the 230°C, 120-160°C and 45-100°C heat treatments. On the second axis, CAN 2, the 230°C and 120-160°C treatments reacted similarly, with high loadings on CAN 2. The top ten carbon sources responsible for separation of the groups are presented in Table 5, with the loadings of the variables on CAN 1 and CAN 2. The substrates with highest loadings on each canonical discriminant variable were different at one and six months, except for D-xylose and L-asparagine (Table 5).

DISCUSSION

Rapid heating of humus resulted in an altered microbial community structure, the changes being evident two months after the heating treatments and subsequent inoculation. The samples were inoculated with the microflora representing the original community of the humus in order to overcome the sterilizing effect of heat. However, it is not known whether the microbial community in the humus had survived the heat treatment and proliferated or whether it had originated from the inoculum. Labeda *et al.* (1975) have shown that even exposure to 160°C for 3 h is not adequate for complete soil sterilization, so it is likely that at least some part of the original microflora survived our treatments. In general, microbes are more resistant to dry heat than moist heat (Wolf and Skipper, 1994), and in dry soil, as used in our study, they can survive at temperatures clearly higher than those used in autoclaving. In our study, after one month incubation the C_{mic} was fairly similar between samples heated at 45-160°C, indicating that heating *per se* did not prevent microbial proliferation at this temperature range. It might be claimed that the heat-induced changes in microbial community structure can be explained by the differential re-colonization after heating. However, we suggest that the effect of substrate quality (humus) and differences in re-colonization are inter-related, and the substrate quality may govern the re-colonization of microbes when other important environmental factors (moisture, temperature, pH) are controlled.

The altered microbial community structure in the 230°C-treated samples, as indicated by changes in PLFA and substrate utilization patterns can be attributed to the higher pH and the observed changes in the structure of the humus as indicated by the FTIR spectra. An increase in pH is known to cause sharp changes in microbial biomass and its activity, culturability and fatty acid composition (Zelles *et al.*, 1987; Smolander and Mälkönen, 1994; Bååth and Arnebrant, 1994; Bååth *et al.*, 1995). Also the structure of the soil organic matter has been shown to change after prescribed burning or heating at 350°C (Almendros *et al.*, 1990; Pietikäinen and Fritze, 1995; Bååth *et al.*, 1995; Knicker *et al.*, 1996). The ratio of fungal to bacterial PLFAs was unaffected by the temperature treatments. Regardless of the temperature treatment or sampling date it remained at a low level, which is a common phenomenon in long incubation experiments (e.g. Frostegård *et al.*, 1996). We can conclude that the observed changes in microbial communities could be related to changes in the quality of organic matter and pH only in the 230°C-treated samples.

The differences in the community structure and substrate utilization pattern between 45-160°C-treated humus samples were also clear, but not connected to the changes in the quality of organic matter as measured. The FTIR or NMR spectra were not able to characterize changes in the humus heated at this temperature range. The changes occurring during thermal treatment may include oxidation (Bunt and Rovira, 1955; Nussbaum, 1993) or changes in the proportions of hemicelluloses, cellulose and lignin (Fernández *et al.*, 1997). These changes would probably occur only in a small fraction of the humus, and thus be undetectable by spectroscopy of whole humus. The observed changes in microbial community at lower temperatures (45-160°C) could hardly be explained by an alteration in humus pH, since at six months, when the microbial communities of control and 160°C-treated samples differed most clearly, the difference in pH between these treatments was only 0.15 units.

We can try to indirectly deduce reasons for the changes in community structure from certain features of the PLFA profiles, i.e. the *trans/cis* ratio of the isomers of monoenoic fatty acids. In studies using pure cultures, stress factors such as nutrient deprivation (Guckert *et al.*, 1986), desiccation (Kieft *et al.*, 1994) and osmotic stress and high incubation temperature (Heipieper *et al.*, 1996) have been shown to increase the *t/c* ratio. In our study, after one-month incubation the *t/c* ratio of 160°C-treated sample and control were similar, as shown in Fig. 5. At the same time the C_{mic} in the 160°C-treated had recovered well and did not differ from that in the control. During the incubation the *t/c* ratio in the control treatment decreased, while that in the severely heated samples increased. This might indicate starvation in the severely heated samples; desiccation could not be the stress causing agent as the moisture content of the humus was kept constant during incubation. In field studies, an increase in the *t/c* ratio has been observed in peat samples when the decomposition has proceeded to a high degree and available carbon has become limited (Borga *et al.*, 1994). Although it is tempting to describe the changes in *t/c* as *in situ* transformations of *cis* configuration to *trans* in microbial cell membranes, it should be borne in mind, that the altered *t/c* ratio may, in mixed cultures, as well indicate changes in the abundance of species having either *trans* or *cis* monoenoics.

The recovery of microbial biomass in the 160°C-treated sample at one-month

and a steady decline until six months might indicate a flush of easily decomposable carbon released by heating at high temperatures. This substrate would later be used up and the depletion of easily decomposable substrate would lead to decreasing microbial biomass. The observed initial flush is in accordance with the results by Serrasolsas and Khanna (1995), who observed a period of high respiration lasting for 30 d after heating of soil and a subsequent decrease during the second phase: 30-210 d. They described the effect as being a result of the increased amount of soluble carbon depleted during the 30-d incubation. Although part of the initial substrate input originated from killing of organisms by heat, it has been shown that treatments involving heat or irradiation affect soil organic matter directly, rendering it more decomposable (Jenkinson, 1966). Heating has long been known to increase the solubility of organic matter in soil (Salonius *et al.*, 1967; Diaz-Raviña *et al.*, 1992), although losses of organic matter are also apparent.

As summarized by Chambers and Attiwill (1994), the effects of heating on soil properties are highly variable, depending on soil type, moisture and oxygen supply during heating and the actual temperature reached within the soil column. Moist soils are more affected by heating, since the changes occurring in soil are at least partly a consequence of the action of peroxides (Salonius *et al.*, 1967). The temperature range used in our study is obviously encountered also in field conditions. A low intensity fire (like prescribed burning) consumes the humus layer only partly, and thus a layer of unburned humus is left under the uppermost black charcoal. If the humus was already dry before the fire, the situation would be similar to our heating experiment, and the degree of heating be dependent on the heat transfer from the burning fuel. When the humus is moist by the beginning of the fire, the humus starts to evaporate water when its temperature reaches 100°C (Chandler *et al.*, 1983). Thus the heating at < 100°C in moist soil in field conditions is not comparable to our mild heating (45-100°C) of dry humus. When the water was been evaporated the temperature in the humus layer rises rapidly, and temperatures similar to the upper range (120-230°C) used in our experiment can occur in humus. We suggest that the commonly observed low size of microbial biomass after prescribed burning may partly be due to the effect of dry heating on humus, which has the capacity to change the properties of humus and reduce, in the long term, the size of the microbial biomass.

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Paper V

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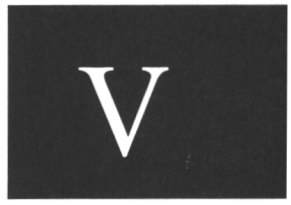

Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus

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Abstract. Wildfires produce a charcoal layer, which has an adsorbing capacity resembling activated carbon. After the fire a new litter layer starts to accumulate on top of charcoal layer, and it liberates water-soluble compounds, which percolate through the charcoal and the unburned humus layer. We first hypothesized that since charcoal has the capacity to adsorb organic compounds it may form a new habitat for microbes, which decompose the adsorbed compounds. Secondly, we hypothesized that the charcoal may cause depletion of decomposable organic carbon in the underlying humus and thus reduce the microbial biomass. To test our hypotheses we prepared microcosms, where we placed non-heated humus and on top one of the adsorbents: non-adsorptive pumice (Pum), charcoal from *Empetrum nigrum* (EmpCh), charcoal from humus (HuCh) or activated carbon (ActC). We watered them with birch leaf litter extract. The adsorbing capacity increased in the order Pum < HuCh < EmpCh < ActC, the adsorbents being capable of removing 0%, 26%, 42% and 51% of the dissolved C_{org} in the litter extract, respectively. After one month, all adsorbents harboured microbes, but their amount and basal respiration was largest in EmpCh and HuCh, and smallest in Pum. In addition, different kinds of microbial communities in respect to their phospholipid fatty acid and substrate utilization patterns were formed in the adsorbents. The amount of microbial biomass and number of bacteria did not differ between humus under different adsorbents, although different microbial communities developed in humus under EmpCh compared with Pum, which is obviously related to the increased pH of the humus under EmpCh, and also ActC. We suggest that charcoal from burning can support microbial communities, which are small in size but have a higher specific growth rate than those of the humus. Although the charcoal layer induces changes in the microbial community of the humus, it does not reduce the amount of humus microbes.

Introduction

During forest fire or prescribed burning a certain proportion of the vegetation, litter, coarse woody debris and soil organic layer is combusted, the degree of burning being dependent on abiotic factors like weather conditions, moisture, and amount and distribution of fuel (Johnson 1992). If combustion of organic matter is complete, only the inorganic nutrients previously incorporated in dead or living plant tissue are left on the burned ground as a layer of white ash. On the contrary, a lightly burned area is characterized by charred remnants of litter and plant material, and the burned area is covered with a layer of black charcoal (Chandler et al. 1983). The inorganic nutrients liberated due to burning have been widely studied, as their release in soil is responsible for many of the changes in tree seedling growth or agricultural crop

production found on burned soils (reviews by e.g. Ahlgren and Ahlgren 1960, Viro 1974, Chandler et al. 1983). However, the black carbon produced by vegetation fires is receiving growing attraction as it is highly resistant to decomposition and occurs in trace amounts in air, water, soil and sediments (Tolonen 1986, Rose 1990, Cofer et al. 1996).

The active role of charcoal as an adsorbing agent in northern coniferous forests was lately revealed by Zackrisson et al. (1996). They showed that charcoal acts like activated carbon and adsorbs allelopathic compounds liberated by *Empetrum hermaphroditum* (crowberry). If these allelopathic phenolics were not adsorbed by the charcoal, they would hinder the establishment of tree seedlings (Nilsson and Zackrisson 1992, Zackrisson and Nilsson 1992). Thus, on nutrient poor sites, where *E. hermaphroditum* tends to monopolize the ground vegetation, the forest regeneration is enhanced by a regular fire cycle, which constantly produces and activates charcoal.

Since the charcoal layer is capable of adsorbing phenolics, we presumed that it also can adsorb other organic compounds (e.g. carbohydrates, amino acids and organic acids leached from vegetation and litter) from the soil water, resembling the action of activated carbon in e.g. waste water treatment, where biological growth occurs on the surfaces of activated carbon (Rodman et al. 1978). On top of the charcoal layer formed during a moderate fire, a layer of new litter starts to accumulate originating from the established vegetation. The freshly formed litter is rich in water soluble compounds, which are readily leached by precipitation (Aber and Melillo 1991 p. 184), and percolated through the underlying charcoal layer, as substantial amounts of soluble carbon are shown to be redistributed in the soil profile by water (Guggenberger and Zech 1994). In boreal coniferous forest the pioneer tree species after fire are deciduous trees; silver birch (*Betula pendula* Roth), downy birch (*B. pubescens* Ehrh.), rowan (*Sorbus aucuparia* L.) and grey alder (*Alnus incana* (L.) Moench) (Lindholm and Vasander 1987). These post-fire tree species produce leaf litter, from which the water-soluble compounds are more easily leached than from needle litter of pine or spruce (Nykvist 1963, Johansson 1995). The water-soluble compounds include e.g. carbohydrates, amino acids and phenolics (Palm and Rowland 1997), and their proportion from leaf dry matter varies between 10 and 32% depending on both biological and methodological differences (Viro 1955, Nykvist 1963, Berg and Wessén 1984, Johansson 1995). These substances serve as an energy rich carbon source, and are preferentially decomposed by soil microbes (Nykvist 1963).

After a fire a more or less heated humus layer can be found under the charcoal layer. The microbial biomass in the underlying layer reduces after burning (Pietikäinen and Fritze 1995, Prieto-Fernández et al. 1998), and the microbial community changes, which can be seen as a reduced proportion of fungi and an increased proportion of bacteria, especially actinomycetes (Bååth et al. 1995). The post-fire recovery of the humus microflora, under the charcoal, to the preburn levels is remarkably slow (Fritze et al. 1993, Prieto-Fernández et al. 1998), and may be a consequence of the overlying charcoal, which might reduce the amount of easily decomposable substrates in the percolating water and cause substrate depletion to microbes in the underlying humus.

We hypothesize first that since charcoal has the capacity to adsorb organic

compounds, it may form a new habitat for a microbial community, which utilizes the adsorbed compounds as substrate. Secondly we hypothesize that the charcoal may negatively influence the microbial community of the underlying humus layer by capturing soluble carbon compounds from the percolating water. This, in turn, might limit the availability of dissolved organic carbon to the microbes of the underlying humus.

To test our hypotheses we prepared microcosms with humus and charcoal resembling the natural situation after a fire. On the bottom we placed non-heated humus and on the top either an equal amount of charcoal, or control material. We incubated the microcosms for one month and watered them with a filtered litter extract, which was prepared from newly fallen birch leaves. After the incubation we examined the microbial biomass and activity, community structure and substrate utilization pattern together with growth rate of bacteria in the humus and the charcoal, and in the control material.

Materials and methods

Preparation of microcosms, charcoal and litter extract

The experiment was carried out in microcosms, which were prepared from 1-liter polyethene bottles (inner diameter 8.1 cm). The bottom of the bottle was cut off and the bottles were hung upside down in an incubator. The microcosm was left open so that water could drain through. The microcosms were assembled by placing fresh humus equal to 25 g dry matter (d.m.) on the bottom of the vessel over a graticule. On the top of the humus, separated by a piece of polyester fabric (mesh size 250 µm), we placed 25 g d.m. of one of the adsorbents to be tested. The quantity (25 g per 51.5 cm^{-2} = 4.8 kg m^{-2}) and the thickness (4-5 cm) of the humus in the microcosm corresponds to values found in *Vaccinium myrtillus* (L.) type forests for quantity (4-6 kg m^{-2}) and thickness (5 cm) of the humus layer (values calculated from Liski and Westman 1995 and Pietikäinen et al. 1999). Since we wanted to study the potential effect of charcoal on the underlying humus, the quantity (4.8 kg m^{-2}) and thickness (2-3 cm) of the charcoal in the microcosms was an overestimation compared to the amount of charcoal found after a wildfire. The amount of black carbon formed during burning may be up to 3% of the carbon exposed to fire (Kuhlbusch 1999). If the quantity of the humus layer is taken as 5 kg m^{-2}, logging slash 2 kg m^{-2} (Vasander and Lindholm 1985), needles 0.4 kg m^{-2} (Mälkönen 1974) and forest floor vegetation 0.1 kg (Mälkönen 1974) and the C content of all fractions is presumed to be 50%, the maximun amount of C exposed to fire may be 3.25 kg m^{-2}, and accordingly the maximum amount of black C may be ca 100 g m^{-2}. This value fits into the range (98-207 g charcoal m^{-2}) measured by Zackrisson et al. (1996) in soils with different time of succession after fire in northern Sweden. However, since charcoal contains organic carbon as well as black carbon (Kuhlbusch and Crutzen 1995) and also other elements derived from the fuel, the amount of charcoal formed is somewhat higher than the amount calculated for black carbon (100 g m^{-2}). As a result, the amount of charcoal in our experiment was 10-50 times higher than the amount found after a fire under field conditions.

The humus for the experiment was collected in a mixed stand of mature Scots

pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karsten). After removing the moss layer (*Pleurozium schreberi* (Brid.) Mitt.) and the dwarf shrub vegetation (*Vaccinium myrtillus* L.), the forest humus layer (F+H) was removed, transported to the laboratory, and passed through a 2.8-mm sieve. The sieved humus material was stored at 4°C for six months before being transferred to the microcosms. The properties of the humus, with standard error of the mean in the parentheses (n=6), were: percent of dry matter 29.8% (± 0.1), percent of organic matter as loss-on-ignition 81.2% (± 0.3), pH 4.30 (± 0.01) and water holding capacity (WHC) 6.0 (± 0.1) ml g^{-1} d.m. For methods used see the paragraph "Chemical analyses".

The overlying material was either charcoal, or one of the control materials. These will all be referred to as adsorbents. The adsorbents chosen for the experiment were 1) pumice stone (Pum) as a non-adsorbing negative control (Riedel-deHaën), 2) charcoal prepared from *Empetrum nigrum* L. twigs (EmpCh), 3) charcoal prepared from forest humus (HuCh) and 4) commercially manufactured granular activated carbon (ActC) as a positive control (YA-Kemia, Helsinki, Finland). EmpCh and HuCh were combusted in a muffle furnace at 450°C for 30 min, and after cooling down, they were passed through a 0.6-mm sieve (for preparation of charcoal see also Zackrisson et al. 1996). The four different adsorbents were applied in four replicates, which resulted in 16 microcosms.

For preparing the leaf extract freshly fallen leaf litter was collected in a birch stand dominated by downy birch (*B. pubescens*) with some scattered individuals of silver birch (*B. pendula*). The concentration of glucose, fructose, inositol and sucrose in the birch leaves as extracted by ethanol were 13.5, 10.2, 4.2 and 0.71 mg g^{-1} d.m., respectively (determination described under "Chemical analyses"). The water extract of the leaf litter was prepared by adding ground leaves equal to 200 g d.m. to 20 l water. This ratio of leaves to water corresponds to an annual litter fall of 1500 kg ha^{-1} (Mälkönen 1977, Viro 1955), which is leached by precipitation of 15 mm (rainfall during one week in October in southern Finland) (Finnish Meteorological Institute 1998). The suspension was shaken (200 rev min^{-1}) for 2 h, filtered and stored in 250-ml polyethene bottles at -20°C. The extract contained 730 mg l^{-1} organic carbon (C_{org}) and 170 mg l^{-1} glucose and its pH was 5.2.

Before starting the incubation of the microcosms, we tested the capacity of the four adsorbents to bind C_{org} and glucose in the birch leaf litter extract and a glucose solution. We added the adsorbents (3 g) to the leaf extract, a glucose solution (1000 mg l^{-1}) or water (volume of all solvents 50 ml) using two replicates in each combination, shook at 200 rev min^{-1} for 2 h, filtered to remove the adsorbent (filter mesh: 0.45 µm) and measured the remaining concentration of C_{org} and glucose in the solvents as described later.

Before adding the leaf litter extract to the microcosms it was melted and filtered through a 0.45 µm membrane (GHP, Gelman Sciences) in order to reduce the amount of microbial cells being added to the microcosms. Only on the first occasion were the microcosms moistened with an unfiltered extract to provide them with a microbial inoculum. The material for the experiment was collected near the field station of the Finnish Forest Research Institute in Ruotsinkylä, Tuusula in southern Finland (60°20'N, 25°00'E).

Incubation

During three days the two-layer microcosms were gradually moistened with the unfiltered leaf extract until the extract drained through the microcosms (to water saturation point). Then they were placed in random order in an incubator at 20°C, 60% relative humidity in the dark for 29 d, and during the incubation, they were moistened with the 0.45-µm filtered leaf extract three times a week. The extract was always added until it was seen to drain through, approximately 35 ml per microcosm being adequate. After the 29-d incubation the microcosms were destructively sampled; adsorbents and humus samples were placed in separate polyethene bags and stored at +4°C. All the microbiological analyses were started within one week after the sampling.

Microbiological analyses

Microbial biomass carbon (C_{mic}) was determined from humus and adsorbent subsamples equal to 2.0 g d.m. using the substrate-induced respiration (SIR) method as described by Priha and Smolander (1994) and based on the theory of Anderson and Domsch (1978). The water holding capacity (WHC) values, which were needed for adjustment of optimum moisture, were 1.0, 2.9 and 1.5 ml g^{-1} d.m. for pumice, charcoal (both EmpCh and HuCh) and activated carbon, respectively. Basal respiration was measured from separate subsamples also equal to 2.0 g d.m. as described by Pietikäinen and Fritze (1995).

The ^{3}H-thymidine incorporation technique was used to measure the growth rate of bacteria released from the humus and the adsorbents. The measurement was done based on the protocol developed for soil samples by Bååth (1992), with the details described by Pennanen et al. (1998). The bacterial suspension for the assay was prepared by adding fresh humus equal to 1.5 g d.m. or adsorbent equal to 2.0 g d.m. to 100 ml purified water. The suspension was shaken (250 rev min^{-1}) at +4°C for 1 h, centrifuged (10 min, 750 x *g*) and the supernatant was filtered through quartz wool. A 2-ml portion of each suspension was incubated for 2 h in 100 nM [methyl-^{3}H] thymidine (925 GBq $mmol^{-1}$, Amersham) at 22°C. The labeled thymidine incorporated in the bacterial cells (total macromolecules) was measured with a Wallac 1411 liquid scintillation counter. The ^{3}H-thymidine incorporation was calculated as mol TdR g^{-1} d.m. h^{-1}, where TdR refers to deoxythymidine. For calculating the specific incorporation rate the bacterial cells in the incubation suspension were counted after staining with acridine orange (AO). The diluted suspension was filtered on a black 0.22 µm polycarbonate membrane (Osmotics Inc.) and stained with 0.1% AO for 3 min. The stained cells were counted under a Leitz Laborlux S epifluorescence microscope using a counting grid (40 µm x 40 µm) and a magnification of x1000.

The phospholipid fatty acids (PLFA) present in the cell membranes of the microbes were determined from moist humus subsamples (0.5 g) and adsorbents (4.0 g) using the method and nomenclature described by Frostegård et al. (1993). PLFAs are essential membrane components of all living cells and they are not found in storage compounds or in dead cells. As specific types of PLFAs predominate in different microbial subgroups, changes in microbial community structure can be

detected by changes in the PLFA patterns (Zelles 1999).

The substrate utilization pattern was analyzed using Biolog Ecoplates® (Biolog, Inc., Hayward, California, USA), which contain 31 different substrates + water. The suspensions prepared for the thymidine incorporation assay (1.5 g d.m. humus + 100 ml water and 2.0 g d.m. adsorbent + 100 ml water) were used for inoculating the Biolog plates. The suspension was diluted 1:10 and the wells of the plates were filled with 150 µl. After inoculation the plates were incubated in the dark at 20°C and 35% relative humidity for seven days. The absorbances of the plates were recorded once a day (actual reading times: 23, 37.5, 62, 88, 113, 134.5 and 162 h) with a Labsystems Multiscan MS plate reader using a 590-nm filter.

Chemical analyses

From the humus and adsorbents the pH was measured in water suspension (sample:water 3:5 v/v). Water content was determined after drying portions of the samples overnight at 105°C. The amount of organic matter was determined as loss-on-ignition by heating at 550°C for 4 h and weighing the remaining mineral material. Total carbon and nitrogen were determined by dry combustion with a Leco CHN-1000 analyzer.

The concentration of glucose in the birch leaf extract was determined from a 5-ml portion, which was first lyophilized. The dried material was dissolved in 2 ml 80%-ethanol, which contained phenyl-β-D-glucoside (1.0 mg ml^{-1}) as the internal standard (Marcy and Carroll 1982). A 0.5-ml portion of the solution was removed and dried under nitrogen. The silylation was done by dissolving the dried sample in 400 µl of 21% TMSI (2.1 g 1-(trimethylsilyl)-imidazole in 10 ml pyridine) (Knapp 1979). The concentration of glucose was measured using a Hewlett Packard 6890 gas chromatograph connected to a mass selective detector HP 5973. The temperature of the injector was 260°C and the column temperature was raised from the initial 110°C with a rate of 10°C min^{-1} to a final temperature of 300°C. Helium was used as carrier gas with a flow of 25 ml min^{-1}. The concentrations of glucose, fructose, inositol and sucrose in the birch leaves was measured from 0.5 g dried, ground leaves using the same protocol as for the leaf extract.

Dissolved organic carbon was measured from 0.45-µm filtered litter extracts with a Shimadzu TOC-5000 total organic carbon analyzer.

Data analyses

All the results were calculated per dry matter of humus or adsorbent. The number of AO stained bacteria was log transformed to enable similar scales in the ordinate in Figs 1e and 1f. The differences in measured variables and scores of the principal components (PC) between the treatments (i.e. adsorbents) were detected using one-way ANOVA followed by Tukey's test separately for the adsorbents and humus samples. The individual PLFAs were expressed as mole percentage (mol%) of the total PLFA content of the sample. The mol% values for PLFAs 16:1ω7t and 16:1ω7c were used to calculate the ratio of the *trans* and *cis* configurations of 16:1ω7 (i.e. the *t/c* ratio). For the Biolog data individual substrates were corrected for background absorbance by subtracting the absorbance of the control (water) well.

The corrected absorbance values of the subsequent readings were used to calculate the total area under the absorbance curve (Sharma et al. 1997). The calculated values (area under the curve) were added together for each sample and the proportions of individual substrates from this total area were expressed as area%. The area percentages from the Biolog data as well as the mol% values from PLFA analysis were subjected to principal component analysis (PCA) using the correlation matrix. The ANOVA and PCA were performed using Statistix 4.1 (Analytical Software, St. Paul, MN, USA) and Unscrabler 6.1 (Camo AS, Trondheim, Norway) statistical packages, respectively.

Results

Adsorbents

Prior to the incubation the potential capacity of the adsorbents to bind organics during 2 h in various solutions (leaf litter extract, glucose or water) was tested. The adsorbing capacity of the adsorbents increased in the order Pum < HuCh < EmpCh < ActC, the adsorbents being capable of removing 0%, 26%, 42% and 51% of the dissolved C_{org} in the leaf litter extract, respectively (calculated from data in Table 1). The adsorbents did not release glucose into water, but ca. 90 mg l^{-1} C_{org} was rendered soluble from HuCh and obviously also EmpCh when exposed to water.

After the 29-d incubation the pH value differed significantly ($P<0.001$) between all adsorbents. The pH was lowest in ActC (5.5) and it increased to the maximum value of 6.9 in the order: ActC < EmpCh < Pum < HuCh (Fig. 1a). All four adsorbents harboured microbes, but the amount of C_{mic} and bacterial cells differed significantly between adsorbents (C_{mic} $P<0.001$, cells $P=0.013$). The amount of C_{mic} and number of bacterial cells were lowest in Pum, while the highest values were found in EmpCh (Fig. 1c,e). The amount of C_{mic} in HuCh and ActC was somewhat lower than that in EmpCh, but still three times the amount found in Pum. On the average, the amount of microbes in the adsorbents (Fig. 1c,e) was only a minor fraction of that found in humus (Fig. 1d,f), the amount of C_{mic} and the number of bacterial cells in the adsorbents being 15% and 2.2% of the corresponding value in humus, respectively.

Basal respiration had low values in Pum and ActC, while the level in both charcoals (EmpCh and HuCh) was 3-4 times the value measured in Pum and ActC (Fig. 1g). A similar response pattern was seen also when measuring the TdR incorporation rate with EmpCh and HuCh showing the highest rates of TdR incorporation (Fig. 2a). However, when the TdR incorporation rate was divided by the total number of bacterial cells (i.e. specific growth rate), the differences between the adsorbents became non-significant (Fig. 2c).

Totally different kinds of microbial communities were formed in the four adsorbents judged by their PLFA patterns (Fig. 3). The microbial community inhabiting Pum was clearly separated on PC1 from microbial communities inhabiting the other adsorbents (scores of PC1 $P<0.001$). The microbes in Pum were characterized by a high percentage of cy19:0, 20:4, 18:1ω9 and cy17:0 (Table 2). Microbes inhabiting EmpCh and ActC got similar scores on PC1 both microbial communities being relatively rich in e.g. 14:0, i16:0, 17:1ω8 and a17:0. However,

Table 1. *Remaining concentrations of dissolved organic carbon and glucose in birch leaf litter extract, a glucose solution of 1000 mg l^{-1} and water after exposure to one of the adsorbing agents. Pum refers to pumice, an inert control material. EmpCh and HuCh refer to the charcoal prepared from Empetrum nigrum twigs and the humus (F+H) of a coniferous forest, respectively. ActC is commercially manufactured activated carbon.*

	Remaining dissolved organic C (mg l^{-1})		Remaining concentration of glucose (mg l^{-1})		
Adsorbent	Litter extract	Water	Litter extract	Glucose solution	Water
Initial solution	730	3	170	1000	0
Pum	750	3	n.d.*	n.d.	n.d.
EmpCh	420	n.d.	120	890	0
HuCh	540	90	150	990	0
ActC	360	4	20	480	0

* n.d. = not determined

the microbial community of ActC differed ($P<0.001$) from the communities in the other adsorbents on PC2. PLFAs with the highest negative loadings on PC2 (abundant in EmpCh and also HuCh) were 16:0 and 10Me18:0. The microbes in ActC, which were clustered in the opposite end of PC2 were relatively richer in 19:1a, 18:1ω7, i15:0 and 20:0 than those in EmpCh and HuCh. The ratio of *trans* and *cis* configurations of the PLFA 16:1ω7 in the microbes living in the adsorbents was on average 0.058 ± 0.004, with no significant differences between adsorbents.

The microbial communities in the adsorbents differed in respect to their substrate utilization patterns as well (Fig. 4, Table 3). PC1 explained 27% of the variation in the data, and the scores of ActC and EmpCh were significantly different ($P=0.006$) on the first principal component. PC2 explained 13% of the variation, and it separated the treatments Pum and HuCh from ActC ($P=0.006$). In Fig. 4 we have deliberately plotted the scores of PC2 on the abscissa and correspondingly the scores on PC1 on the ordinate, since in separation of the microbes in Pum and ActC was based on the degradation of substrates forming PC2 rather than PC1. The separation of ActC and EmpCh, which for the PLFA data was seen on PC2, was, in the case of substrate utilization patterns, accordingly on PC1. The substrates with the highest absolute loadings on PC1 and PC2 are shown in Table 3.

Table 2. *The ten most highly weighted phospholipid fatty acids (PLFA) forming the principal components (PC). No significant differences between treatments in microbial communities of humus existed on other principal components, so only the loadings for PC2 are shown.*

Microbes of adsorbents				Microbes of humus	
PC1		PC2		PC2	
PLFA	Loading	PLFA	Loading	PLFA	Loading
cy19:0	-0.286	16:0	-0.372	16:1ω5	-0.316
20:4	-0.267	10Me18:0	-0.369	20:5	0.308
14:0	0.257	19:1a*	0.333	cy17:0	0.301
18:1ω9	-0.244	18:1ω7	0.284	16:1ω9	-0.294
i16:0	0.241	i15:0	0.264	20:0	0.292
17:1ω8	0.237	20:0	0.234	cy19:0	0.291
a17:0	0.236	i14:0	-0.224	16:1ω7c	-0.291
i14:0	0.234	a15:0	-0.223	i17:0	-0.273
cy17:0	-0.227	a17:0	0.210	10Me18:0	-0.220
a15:0	0.221	cy17:0	-0.202	a15:0	-0.190

* position of double bond not determined

Underlying humus

The one-month incubation and moistening with birch leaf litter extract (pH value 5.2) resulted in a higher pH ($P<0.001$) in humus under EmpCh and ActC, while under Pum and HuCh the pH was 0.8 units lower (Fig. 1b). The amount of C_{org} and total nitrogen in the humus did not differ between the treatments or compared to the values measured from the humus before the start of the experiment (data not shown). The amount of C_{mic} in the humus under different adsorbents varied from 6.1 to 7.0 mg g^{-1} d.m., and the number of bacterial cells varied from 2.0 to 2.4 x 10^{10} cells g^{-1} d.m. No significant differences between treatments were detected for either variable (Fig. 1d,f). The basal respiration activity was significantly higher ($P=0.001$) under EmpCh and ActC compared to Pum and HuCh (Fig. 1h). The bacteria inhabiting humus under ActC had significantly higher ($P=0.004$) thymidine incorporation rate than bacteria under EmpCh and HuCh (Fig. 2b). The specific TdR incorporation rate of the bacteria in the humus was very low compared to that in the adsorbents (Fig. 2c,d).

Table 3. *The ten most highly weighted substrates forming the principal components (PC) which contribute to the separation of treatments. Since the microbial communities in the humus did not differ between treatments (adsorbents on top) on PC2, only the loadings for PC1 are shown.*

Microbes of adsorbents				Microbes of humus	
PC2		PC1		PC1	
Substrate	Loading	Substrate	Loading	Substrate	Loading
Phenylethylamine	-0.382	D-Galacturonic acid	-0.288	Glycogen	0.290
α-D-Lactose	-0.329	γ-Hydroxybutyric acid	-0.287	Itaconic acid	-0.274
D-Cellobiose	0.301	i-Erythritol	0.272	D-Galacturonic acid	-0.266
Glycogen	0.278	4-Hydroxy-benzoic acid	-0.270	Glycyl-L-glutamic acid	0.260
L-Serine	-0.258	D-Galactonic acid γ-lactone	-0.269	α-Keto butyric acid	0.254
α-Cyclodextrin	0.221	D-Malic acid	-0.262	α-Cyclodextrin	0.242
N-Acetyl-D-glucosamine	-0.215	D-Xylose	0.240	4-Hydroxy-benzoic acid	-0.235
Itaconic acid	0.214	2-Hydroxy-benzoic acid	0.226	D-Galactonic acid γ-lactone	-0.234
4-Hydroxy-benzoic acid	0.197	D,L-α-Glycerol phosphate	0.217	L-Serine	-0.231
Tween 80	0.189	L-Phenylalanine	0.215	Pyruvic acid methyl ester	0.229

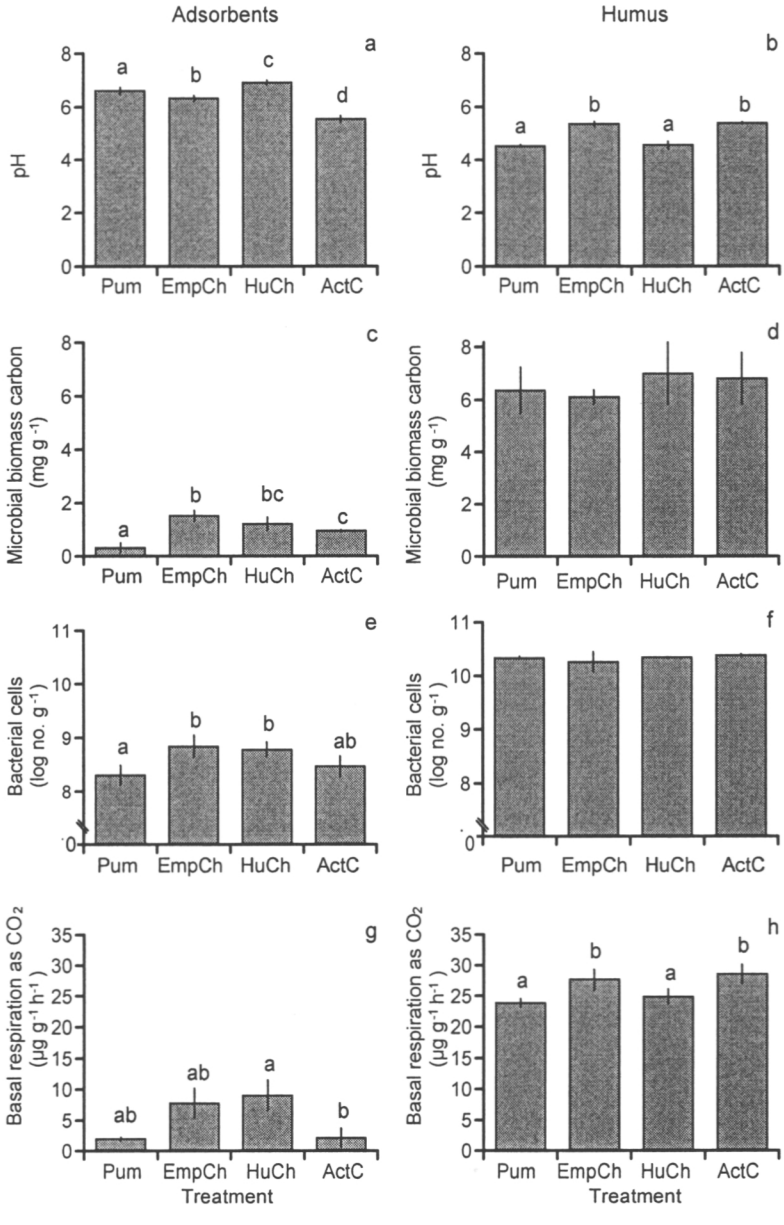

Fig. 1. Characterization of the adsorbents (figures on the left column; a, c, e, g) and humus (figures on the right column; b, d, f, h) after 29-day incubation at 20°C and regular moistening with filtered birch leaf litter extract. Results are calculated per dry matter of the adsorbent or humus. The columns marked with different index letters are significantly different at $P<0.05$; the columns marked with same index letters and columns in figures without any index letters are not significantly different at $P<0.05$ (one-way ANOVA followed by Tukey's test). Error bars represent standard deviation.

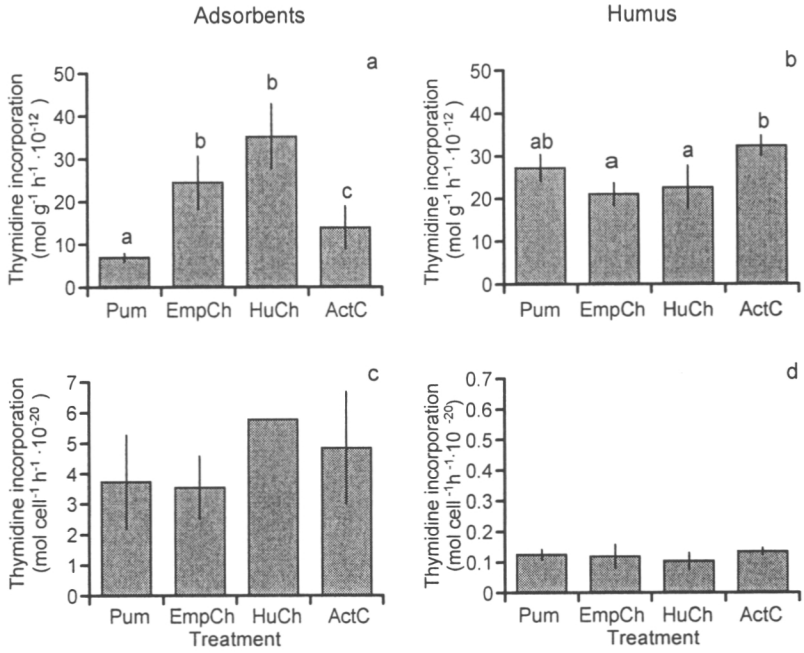

Fig. 2. Bacterial growth rate in the adsorbents (a, c) and humus (b, d) measured as thymidine incorporation rate per sample dry matter (a, b) and as specific growth rate per bacterial cell (c, d). Significance letters and error bars as in Fig. 1. Note the 10-fold difference in the scales between (c) and (d).

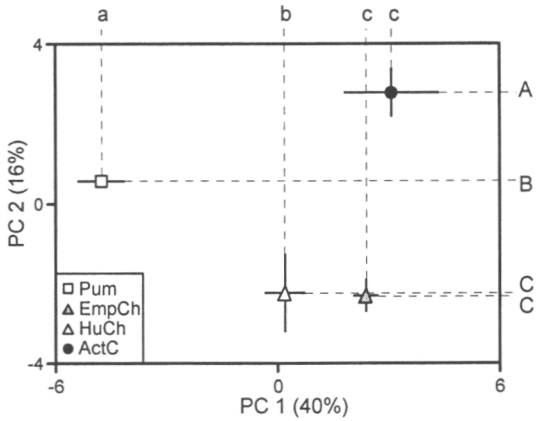

Fig. 3. Phospholipid fatty acid patterns of the microbes inhabiting the four adsorbents. The scores of the first (PC1) and second (PC2) principal components were subjected to one-way ANOVA followed by Tukey's test; and the sample points marked with same index letter along abscissa or ordinate (capital letters) do not differ at $P<0.05$. The percentage of variation explained by each principal component is given in parentheses. The error bars represent standard deviation.

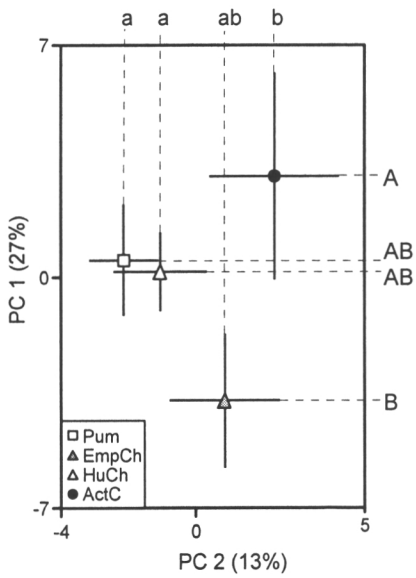

Fig. 4. Substrate utilization patterns (Biolog Ecoplates®) of the microbes inhabiting the adsorbents. Note that the scores of PC1, explaining 27% of variation in the data, are plotted on the ordinate, and scores of PC2 (13%) correspondingly on the abscissa. Significance letters, percentage values and error bars as in Fig. 3.

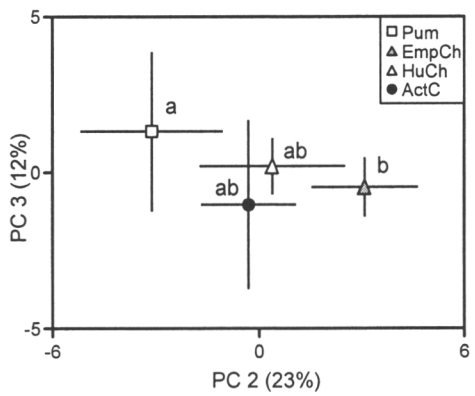

Fig. 5. The phospholipid fatty acid patterns of the microbial communities of the humus under the four adsorbents. PC1 is not shown since it did not contribute to the separation of the microbial communities. The scores of PC2 did not differ between sample points indexed with the same letter (one-way ANOVA followed by Tukey's test) at $P<0.05$. Error bars represent standard deviation.

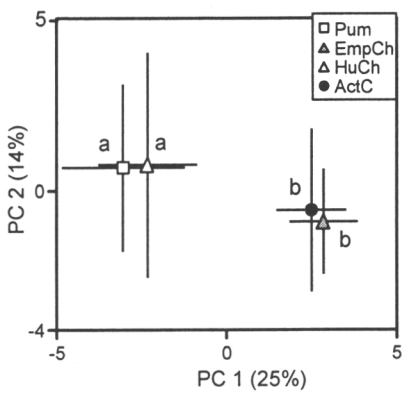

Fig. 6. The substrate utilization patterns of the microbial communities of the humus under different adsorbents. Index letters referring to significant differences on PC1, percentage values, and error bars are as in Fig. 5.

The analysis of the PLFA patterns of the microbial communities under the different adsorbents showed differences between the communities. The microbial communities of humus differed according to the scores of PC2, which explained 23% of the variation in the data (Fig. 5). PC1 did not contribute to the separation of the different treatments. The microbial community inhabiting humus under Pum was negatively weighted on PC2, while the community from the humus under EmpCh was most positively weighted on PC2. When the scores of the samples on PC2 were subjected to ANOVA, a significant difference between communities from humus under Pum and under EmpCh was detected (P=0.004). The communities inhabiting humus under HuCh or ActC were clustered close to each other and did not differ from the other two. The individual PLFAs which got high absolute loadings on PC2 and thus contributed to the separation of EmpCh and Pum are shown in Table 2. There were no significant differences in the *trans/cis* ratio of the PLFA 16:1ω7 between the microbial communities living in the humus under different adsorbents (average *t/c*: 0.115 ± 0.002).

When the substrate utilization patterns of the microbial communities of the humus samples are evaluated, it can be clearly seen, that the communities from humus under EmpCh and ActC were grouped at the opposite end of PC1 compared to communities in humus under Pum and HuCh (Fig. 6), which in turn showed substrate utilization patterns similar to each other (scores on PC1 P<0.001). The loadings of the ten substrates having the highest influence on the separation of Pum+HuCh from ActC+EmpCh on PC1 are shown in Table 3.

Discussion

All the adsorbents tested; pumice, charcoals from *Empetrum nigrum* twigs and forest humus, and commercially manufactured activated carbon, were able to support microbial life when moistened regularly with birch leaf litter extract containing ca. 0.7 g C_{org} l^{-1}. The highest number of bacterial cells was found in EmpCh and HuCh (6.3 - 7.5 x 10^8 cells g^{-1} d.m.) and the lowest in Pum (2.2 x 10^8 cells g^{-1} d.m.). The higher range corresponds to 15 - 20% of the value (4 x 10^9 Pseudomonads g^{-1}) reported by Ehrhardt and Rehm (1985) for a biological activated carbon bed. The larger size of the microbial pool in EmpCh and HuCh than in Pum might be a consequence of the poorer adsorption capacity of Pum (see Table 1) and a lack of attachment surface for microbes. Although the highest adsorption capacity of ActC (Table 1) might imply that also the microbial pool should be largest in ActC, this was not confirmed by the measurement C_{mic} or the number of bacterial cells in the adsorbents. An apparent reason for the lower microbial biomass in ActC, and also Pum, compared to both charcoals can be the lower water holding capacity of the former two (see Materials and methods -Microbiological analyses). The *t/c* ratio, which has been shown to increase under stressing conditions, like desiccation or starvation (Kieft et al. 1994), did not differ between microbial communities of the adsorbents possibly indicating equally good substrate input in all adsorbents.

The most remarkable feature of EmpCh and HuCh was the high basal respiration activity and thymidine incorporation rate per adsorbent dry matter. Moreover, the specific growth rate calculated per bacterial cell in all the adsorbents was one order

of magnitude higher than for bacteria inhabiting the humus. Thus, the substrates of the birch leaf litter extract supported a small but highly active microbial community in the adsorbents, the size of which depended on the properties of the adsorbent. In addition to the size of the microbial community, also its structure was dependent on the properties of the adsorbent, since different kinds of communities developed in all adsorbents judged by their PLFA and substrate utilization patterns. Microbial communities of ActC and EmpCh had similar scores on the first principal component indicating that their PLFA composition was rather similar. The microbial community in Pum differed clearly from the group formed by ActC+EmpCh, while HuCh was situated intermediate. These results show that charcoal from forest fires or prescribed burning can form a new habitat for microbes, which can decompose the dissolved organic compounds present in the percolating water. Obviously, the compounds are first bound on the charcoal before being metabolized by the microbes. A similar mechanism is encountered also in biological activated carbon beds, where microorganisms attach themselves to the external surfaces of the carbon particles and degrade the adsorbed contaminants or other organics (Rice et al. 1978, De Laat et al. 1985, Kim et al. 1997). As a result we can confirm our first proposed hypothesis according to which charcoal may form a new habitat for a microbial community, which at least partly utilizes the adsorbed compounds as substrate. However, it cannot be ruled out that some part of the substrates utilized by the microbial community in the adsorbent might orinate from the humus layer. In this experiment the adsorbent and humus layers were physically in contact with each other, and the transport of substrates from the humus to the adsorbent by e.g. fungal hyphae cannot be estimated.

In our work we did not determine the compounds retained by the adsorbents. Since charcoal has a higher affinity for phenol than for glucose (Rodman et al. 1978) and birch leaves contain water-soluble phenols, the microbes in the adsorbents may have utilized also phenols, not only sugars. However, our aim was to determine the capacity of the four adsorbents to harbour and support microbial communities regardless of their choise of substrate. Glucose was chosen as a model compound in the adsorption test because it is the most abundant sugar in the birch leaves (see Materials and methods) and it is easily decomposed by soil microbes.

The size of the active microbial biomass (measured as SIR), number of bacterial cells, or the *t/c* ratio of the PLFA 16:1ω7 in the humus under the different adsorbents did not differ between treatments. In the separate experiment where the adsorbing capacity of the four adsorbents were tested, the most efficient adsorbent, i.e. ActC, bound 51% of the dissolved organic C in the litter extract (Table 1). This, in turn, would imply that in the incubation experiment, 49% of the C in the litter extract appears to have entered the humus under ActC, and, accordingly, 58% or more has entered the humus under EmpCh, HuCh or Pum. Obviously the microbes in humus received substrates adequately under all adsorbents.

If the humus had been saturated by substrates liberated due to the handling and sieving of the humus, the microbes would have been unable to respond to the slight differences in the substrate input. However, a situation of substrate saturation in the beginning of our experiment is unlikely, as the humus had been stored for six months after sieving, during which the initial burst of substrates has been consumed.

If ActC, EmpCh or HuCh had adsorbed water-soluble compounds from the leaf litter extract during the incubation to a substantially higher degree compared to Pum, the microbial biomass should have been significantly higher or the *t/c* ratio lower in humus under Pum compared to the other humus. Since this was not confirmed by our results, it leads to rejection of the second hypothesis, according to which the charcoal, by adsorbing substrates, may negatively influence the microbes of the underlying humus.

Although no differences in the amount of microbial biomass or bacterial cells between the humus under different adsorbents were detected, the basal respiration activity of the humus under EmpCh and ActC was clearly higher than that under Pum and HuCh. This pattern of response corresponds to that observed for humus pH, with humus under EmpCh and ActC having 0.8 units higher pH than humus under Pum and HuCh. Obviously the higher pH in humus accelerated also respiration activity, as increased soil respiration has been previously demonstrated in e.g. liming experiments, where soil pH has increased (Bååth and Arnebrant 1994). The stimulating effect of ActC and EmpCh on the level of basal respiration in the humus was obviously related to the increased pH under these adsorbents, which in turn could be caused by the adsorbtion of organic acids from the litter extract by the adsorbents and the microbes inhabiting the adsorbents. Thus, this effect may be related to a generally better adsorption capacity of ActC and EmpCh compared to HuCh and Pum (as indicated in Table 1). The stimulatory effect of charcoal is in accordance with the results of Zackrisson et al. (1996) and Wardle et al. (1998), who showed increased SIR in humus under a litterbag filled with charcoal, and in humus collected under a lichen layer (*Cladina* sp.) amended with charcoal in a pot experiment, respectively.

When looking at the structure of the microbial communities inhabiting humus under the different adsorbents, a significant difference in their PLFA and substrate utilization patterns was observed. In both analyses the microbial communities in humus under Pum and EmpCh showed greatest dissimilarity. In the substrate utilization analysis the communities in humus under Pum and HuCh were similar to each other, while communities under ActC and EmpCh formed another coherent group. These groups also coincide with the observed differences in humus pH, indicating that higher pH did not only increase basal respiration activity, but also influenced the substrate utilization patterns of the microbial communities. The separation according to the PLFA data was consistent with the separation based on the substrate utilization capacity for treatments Pum and EmpCh, while adsorbents HuCh and ActC did not induce changes in the microbial PLFA pattern of the underlying humus. A separation pattern resembling that of the humus microbes was also seen for the communities inhabiting the adsorbents. We conclude that the different adsorbents induced changes in the structure of the microbial community of the underlying humus, these changes being evident in basal respiration activity, and structural (PLFA) and metabolic (substrate utilization) profiling. These changes in microbial activity and community structure might be related to the increased pH under Actc and EmpCh.

According to our results, a microbial population might be expected to live in the charcoal layer under the newly formed litter layer in the field. The litter layer would

obviously protect the charcoal microbes from excessive heat and drying. The porous structure of charcoal may offer favorable microsites for the microbes and shelter against soil faunal predators, as proposed by Zackrisson et al. (1996). We suggest that in later studies dealing with fire effects on soil microbes it might be beneficial to include the charcoal layer as a separate sample. However, the charcoal layer itself, or its indirect effects seem not to be responsible for the commonly observed reduction in microbial biomass in the underlying humus after fire.

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