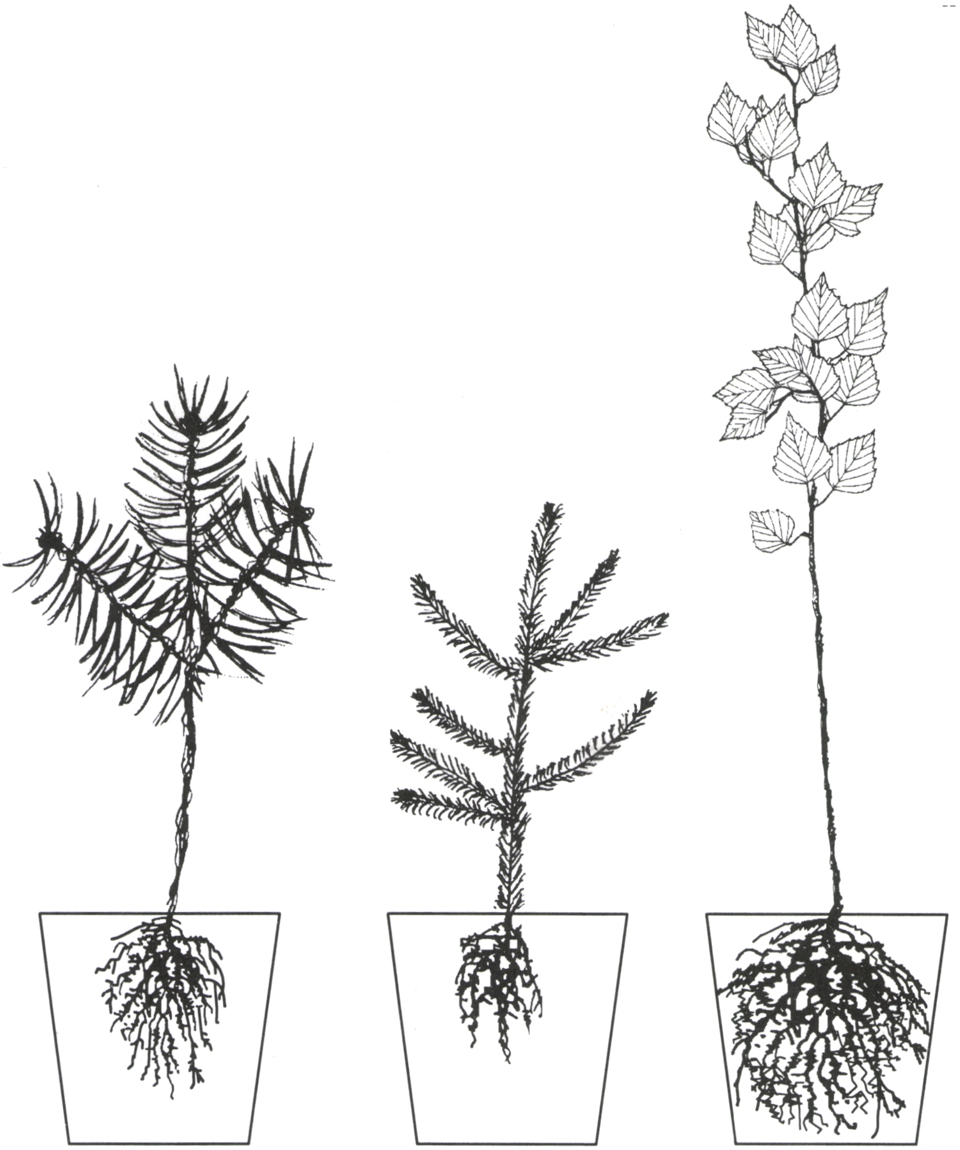
METSÄNTUTKIMUSLAITOKSEN TIEDONANTOJA 731, 1999 30.04.99
FINNISH FOREST RESEARCH INSTITUTE, RESEARCH PAPERS 731, 1999

Microbial activities in soils under Scots pine, Norway spruce and silver birch

Outi Priha

VANTAAN TUTKIMUSKESKUS – VANTAA RESEARCH CENTRE

METLA



METSÄNTUTKIMUSLAITOKSEN TIEDONANTOJA 731, 1999
FINNISH FOREST RESEARCH INSTITUTE, RESEARCH PAPERS 731, 1999

Microbial activities in soils under Scots pine, Norway spruce and silver birch

Outi Priha

Finnish Forest Research Institute
Vantaa Research Centre

Academic dissertation in Environmental Microbiology
Faculty of Agriculture and Forestry
University of Helsinki

To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in Auditorium 2041 at Viikki Biocenter (Viikinkaari 5, Helsinki) on May 21st, 1999, at 12 o'clock noon.

Helsinki, 1999

Supervisor: Docent Aino Smolander

Finnish Forest Research Institute
Vantaa Research Centre

Reviewers: Dr. Heljä-Sisko Helmisaari

Finnish Forest Research Institute
Vantaa Research Centre

Professor Pertti Martikainen

University of Kuopio, Finland
Department of Environmental Sciences
and
National Public Health Institute, Finland
Laboratory of Environmental Microbiology

Opponent: Professor Ken Killham

University of Aberdeen, Scotland
Department of Plant and Soil Science

ISBN 951-40-1678-5
ISSN 0358-4283

Hakapaino Oy, Helsinki 1999

Preface

This work was carried out at the Vantaa Research Centre of the Finnish Forest Research Institute. I would like to thank Professor Eero Paavilainen, head of Vantaa Research Centre, and Professor Eino Mälkönen, head of "Soil department", for providing me these excellent working facilities and the support of Metla.

The work was funded by the Academy of Finland, through the Research Programme of Forest Soil Biology, and the foundations Emil Aaltosen säätiö, Metsämiesten säätiö, and Niemi säätiö, which are all acknowledged.

To my supervisor, Docent Aino Smolander, I am deeply grateful for introducing me into the exciting world of soil microbiology, and for supporting me in the process of becoming a soil microbiologist myself. The doing of this thesis would have been totally different if I wouldn't have always been able to get advice and help from you.

I want to express my sincere gratitude to all of the co-authors of the papers, it has been a pleasure to do co-operation with you. Especially the time in MLURI, Aberdeen, was a refreshing change, and I want to thank Dr. Sue Grayston for welcoming me. Dr. Heljä-Sisko Helmisaari and Professor Pertti Martikainen are greatly acknowledged for reviewing this thesis and giving constructive comments on it.

I have spent a lot of time in Metla while doing this thesis, but it has not been all work: I cannot express with words how much I appreciate getting friends like you, Taina and Laura. Satu M., Satu R., Oili, Hannu, Janna, Jonna and Päivi T.: we have also shared innumerable great moments - both in science and in social life. My thanks go to the whole soil department for a helpful attitude and a nice atmosphere. My special thanks to Anneli, who skillfully helped me in the laboratory work of the last papers and has a great sense of humour. Many thanks go also to Veikko, in addition to advice and help in organic and analytical chemistry also for advice and help in car repairing matters. I appreciate very much Tuula's improving comments on my texts, and Pekka T.'s vast knowledge of soils, which often helped me in replying to reviewers' comments. Thanks to Pirkko R. and Hillevi for typewriting applications at crucial times and taking care of all the paperwork connected to research. My vice-mother Hillevi also deserves thanks as the representative of Metla's Eräkerho, the trips of which are unforgettable!

Thanks Anne - without you many figures of this thesis, especially the seedlings on the front page, would be missing. I am also very grateful for all the people in Keskuslaboratorio who have helped me during these years, especially Merja-Leena and Pirkko H. for all the FIA measurements. And of course thanks to Kevin, Kullervo and Daniel - the impressive GC:s responsible for many results of this thesis.

I have also gotten the pleasure to get to know many great people outside Metla in the world of science, just to mention Aimo, Kim and Pekka V. - thanks for scientific discussions and great dance company in many post-scientific occasions.

There are also people not connected to work whom I would like to thank for being there. My warmest thanks to my family - especially my mother for always supporting me and taking care of my welfare, and my sister Maarit for numerous phone calls sharing thoughts and experiences of the world of research, and of everything else on top of earth. I am extremely grateful to all my friends, especially Päivi, Paula, Susanna and Elina, for still being there and often organizing something to drag me away from roots, tubes and stats! And finally Jussi: my loving thanks to you for being so patient with my ups and downs and for always inventing fun things to do when getting out of work.

Contents

Original publications

This thesis is based on the following articles, which in the text will be referred to by their Roman numerals.

I Priha O. & Smolander A. (1997) Microbial biomass and activity in soil and litter under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at originally similar field afforestation sites. Biology and Fertility of Soils 24: 45-51

II Priha O. & Smolander A. (1999) Nitrogen transformations in soil under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. Soil Biology and Biochemistry 31: 965-977 (in press).

III Priha O., Grayston S.J., Hiukka R., Pennanen T. & Smolander A. Microbial community structure and characteristics of the organic matter in soils under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. Submitted manuscript.

IV Priha O., Lehto T. & Smolander A. (1998) Mycorrhizas and C and N transformations in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings. Plant and Soil 206: 191-204 (in press).

V Priha O., Hallantie T. & Smolander A. Comparing microbial biomass, denitrification enzyme activity, and numbers of nitrifiers in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings with microscale methods. Biology and Fertility of Soils, accepted.

VI Priha O., Grayston S.J., Pennanen T. & Smolander A. Microbial activities related to C and N cycling, and microbial community structure in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings in an organic and mineral soil. Submitted manuscript.

1. Introduction

1.1 Mechanisms by which trees affect soils

Although different tree species tend to establish themselves in different soils, trees also change the soil underneath them. Trees affect the soil by the associated micro-climate that is formed under the tree cover, by their above- and below-ground litter, and by their root activities. These mechanisms affect both the physical, chemical and biological properties of soil.

The trees and their age determine the microclimate that is formed in a certain stand. The degree of shading by tree canopies affects the light and temperature conditions in soil. Precipitation which passes through a forest canopy undergoes both quantitative and qualitative changes; some elements are reduced and some increased. Interception of the precipitation is greater with coniferous than deciduous tree species, but deciduous trees reduce the acid load in wet deposition more than conifers (Hyvärinen 1990). In boreal zones, the tree canopies influence not only the amount of water reaching the soil, but in wintertime also the thickness of snow cover, which in turn affects the depth of soil freezing.

The release of inorganic compounds from litter is a key process affecting nutrient availability and composition of organic matter in soil. Although the amount and composition of above-ground litter of a certain tree species may vary greatly, there are general differences in the structure and decomposability of litters of different tree species. Especially needle and leaf litter differ from each other. A high concentration of lignin in litter decreases its decomposition rate, and lignin : N ratio of litter has been used as a measure of litter quality (Finzi and Canham 1998). In addition to the amount of lignin, the chemical structure and thus its decomposability may differ with different litters (Berg 1986). The waxes of the surface layer and high concentration of lignin and other polyphenolic compounds make needle litter difficult to decompose, whereas leaf litter contains more easily leached and decomposed water-soluble compounds: sugars, amino acids and aliphatic acids (Mikola 1954, Viro 1955, Nykvist 1963, Johansson 1995, Harris and Safford 1996). As with above-ground litter, also the quantity and quality of below-ground litter may vary between different tree species (Finer et al. 1997, Scott 1998), and the concentration of lignin is an important factor in determining its decomposition rate (Berg 1984). Below-ground litter has, however, been studied much less intensively than above-ground litter, and its properties are still largely unknown.

The root activities of the trees also vary with tree species. Smith (1976) showed that the composition of root exudates of *Betula alleghaniensis*, *Fagus grandifolia* and *Acer saccharum* varied significantly. *F. grandifolia* released the largest amount of amino and organic acids, whereas *B. alleghaniensis* released the largest amount of carbohydrates. Not only the rates and patterns of exudation, but also the rates and patterns of nutrient and water uptake may vary between roots of different tree species. The possible differences between root activities of different tree species are currently, however, not well understood.

In addition to these direct effects of trees, they affect the soil indirectly. The above mentioned effects may partly determine the cover and species of understorey vegetation that is established in a stand, which in turn has its own effect on soil (e.g. Chang et al. 1996, Saetre et al. 1997). Ericaceous species often contain high amounts of phenolic compounds and terpenoid resins, whereas herbaceous species contain relatively low amounts of these compounds (Barford and Lajhta 1992, Gallet and Lebreton 1995).

Last but not least, with these mechanisms trees have a strong influence on the soil microbial populations and thus the decomposition rate in forest soil. Concentrations of microbial biomass C and N of total soil C and N were, on average, lower under conifers (white spruce and balsam fir) than under deciduous species, paper birch and trembling aspen (Bauhus et al. 1998). Species specific effects on ammonium and nitrate production and uptake between Douglas-fir, western hemlock and western redcedar were found by Turner et al. (1993). The rates of nitrification and nitrate uptake were highest under redcedar, whereas under hemlock and Douglas-fir low levels of nitrification prevailed.

1.2 Birch versus conifers

The major tree species in Finland are Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) Karst.), downy birch (*Betula pubescens* Ehrh.) and silver birch (*Betula pendula* Roth). Finnish forest soils are naturally acidic because of a base-poor parent material, cool and humid climate, and vegetation which further increases acidification. The most common soil type is podsol, which is characteristic in cool and humid areas. The soil texture of a majority of forest sites is till, of which fine sandy till is most common (Aaltonen 1941). The soil characteristics largely determine the fertility of the site, and also the understorey vegetation which establishes on a stand. The forest site fertility classification used in Finland is based on the understorey vegetation of the site (Cajander 1949). As mentioned earlier, different tree species tend to establish at certain site types. For instance Scots pine is able to grow on nutrient-poor sandy types, whereas spruce is more demanding, and tends to establish at more fertile sites (Eyre 1968). Deciduous trees can be even more demanding regarding site fertility. Nevertheless, pine, spruce and birch can also grow at sites of the same fertility, and may influence the soil characteristics differently.

Birch has a reputation in forestry history as a tree species that improves soil conditions, especially compared to spruce. Studies have indeed shown that when birch cover is developed, soil pH, nutrient contents and earthworm populations may increase, C:N ratio decrease, and mor humus give way to mull (Gardiner 1968, Miles and Young 1980, Mikola 1985). Also soil microbes appear to be stimulated by birch. The decomposition of cellulose was more active in soil under birch than under spruce at originally similar sites (Mikola 1985). Nohrstedt (1985, 1988) found that birch, but not spruce and pine, stimulated nitrogen-fixation by free-living microorganisms in the soil, most of the fixation activity being concentrated in older leaf litter. The densities of *Frankia*, the N_2-fixing root nodule symbiont of *Alnus*, were surprisingly high in soil under birch, in some cases even higher than in soil under the host plant (Smolander 1990). Norway spruce, in turn, has been found to lower the pH, concentrations of exchangeable nutrients and decomposition rates and enhance podsolisation (Nihlgård 1971, Mikola 1985, Binkley and Valentine 1991, Ranger and Nys 1994). Scots pine has not been included in these studies, but white pine was an intermediate between Norway spruce and green ash regarding the effects on soil pH and nutrient contents in the study of Binkley and Valentine (1991).

The reasons for differences in soils under spruce and birch are probably due to several factors, discussed in paragraph 1.1. Ecological conditions (climatic factors) are usually more favourable in deciduous than in coniferous stands. The canopy of birch has a smaller shading effect than that of spruce, which results in a higher temperature and gives more light to the birch stands. Frost in wintertime is stronger under spruce than under birch because of a less even snow cover. Birch leaf litter has a larger content of water-soluble substances and simple carbohydrates and also a somewhat higher pH and concentration of base compounds than coniferous needle litter (Mikola 1954, Viro 1955, Nykvist 1963, Berg and Wessén 1984, Johansson 1995). In addition to soil microbes, birch litter and soil have been shown to favour the presence of earthworms (Huhta 1979, Saetre 1998), which in turn have been shown to enhance decomposition of litter and organic matter and improve the growth of birch seedlings (Haimi and Huhta 1990, Haimi et al. 1992). It has also been suggested that the beneficial effect of birch may arise from the activities of its roots, especially the large amount of labile C released to the soil (Bradley and Fyles 1995).

Miller (1984), on the basis of available data from the literature, developed models to determine whether birch has a soil-improving effect, but found that nutrient cycling in birchwoods is comparable to that in forests of other species with similar rates and patterns of growth. Nevertheless, the effect on soil properties by tree species can be different if relative tree growth rates differ (Alban 1982), and the varying growth rates and patterns can be regarded as a part of the effects of different tree species. Hobbie (1992), suggested that plants from low-nutrient environments grow slowly, produce poor-quality litter and use nutrients effectively, whereas plant species from nutrient-rich ecosystems, like birch, grow rapidly, produce readily degradable litter and further enhance nutrient cycling. It is possible, however, that other fast-growing broad-leaved trees could have similar effects as birch on soil properties.

On the basis of computer simulation models that include the effects of litter quality, Pastor et al. (1987) suggested that the depression of soil N availability by litter from black and white spruce may directly lead to spruce decline. Spruce litter depresses soil N availability because it decays and mineralizes N slowly as a result of its high lignin and polyphenolic contents and low N content. There are also differences between coniferous needle litters. Although nutrient concentrations were higher in spruce needle litter than in pine needle litter, the higher lignin content of spruce needle litter made it more difficult to decompose than pine needle litter (Johansson 1995).

Another question is whether these changes in soil should be called "improving" or "degrading" the soil. Binkley (1995) concludes that no association between soil acidification and nutrient availability is apparent; the availability and turnover of N and P have not followed patterns of soil acidification in experiments, where trees have been planted in similar soil. Indeed, a large part of the data regarding the effects of tree species on soils is derived from trees growing in different parent materials. Solid conclusions concerning the effects of tree species on soil can only be made based on the so called common garden experiments, that is, at sites where trees have been established adjacent to each other in originally similar soil (Binkley 1995). Such studies with different tree species, especially boreal ones, are relatively scarce.

There has been increasing interest in forests of mixed species, which further complicates the evaluation of the influence of different tree species on soil. The effects of mixed-species cannot be extrapolated from the effects observed from single species. In a study of Chapman et al. (1988), nutrient availability and tree growth were enhanced in Norway spruce / Scots pine and depressed in spruce / alder (*Alnus glutinosa*) and spruce / oak (*Quercus petraea*) mixtures compared with single-species stands. The enhanced growth of spruce and pine in mixture was associated with higher than expected rates of respiration, faunal populations and mineralization of N and P, compared with pure stands.

1.3 Rhizosphere as a habitat for microbes

The definitions of rhizosphere differ, but perhaps the most common is that used by Hiltner already in 1904 (see Grayston et al. 1996): the volume of soil adjacent to and influenced by plant roots. Roots affect many physicochemical factors in soil. The pH in the rhizosphere can differ even by 2 pH units from that in the bulk soil; for instance a drop in the pH occurs with plant NH_4-uptake and a rise with NO_3-uptake (Nye 1981, Wang and Zabowski 1998). The uptake of nutrients and water by roots affects, in addition to pH, the moisture and nutrient status, redox potential and aeration of the soil, which all influence soil microbes. Roots also have an important role in developing and retaining the soil aggregate structure, which gives soils protection from wind and water erosion and maintains pores for the storage of water and transmission of water and air (Tisdall and Oades 1982, Miller and Jastrow 1990). The size distribution and concentration of organic matter of soil aggregates may vary with different tree species (Scott 1998).

Trees allocate up to 40-70% of their photosynthetically assimilated C below ground, and of this amount from 2 to 10% is lost as root exudates (reviewed by Grayston et al. 1996). The organic C input by growing plant roots is termed rhizodeposition and can be defined into several groups depending on the chemical nature of the compounds and mode of release. Water-soluble exudates comprise of low molecular weight substrates, like sugars, amino acids, organic acids, hormones and vitamins, and are lost passively without the involvement of metabolic activity. Gases, such as ethylene and carbon dioxide, are often considered as constituents of exudates. Secretions depend on metabolical processes for their release, and are higher molecular weight substances. Lysates, like sloughed-off cells, and mucilage, which covers the roots of many plants and are composed mainly of polysaccharides and polygalacturonic acids, are also constituents of rhizodeposition. The main zones of exudation and secretion are towards the root tip, although also older parts of roots exude significant quantities of organic compounds (Bowen and Rovira 1991).

As suitable C substrates are considered to be the factor most limiting to microbial growth in soil, this extra C usually causes increased microbial biomass and numbers in the rhizosphere (Wardle 1992). The exudates may also act as primers for the degradation of existing soil organic matter (Helal and Sauerbeck 1983, 1986). The marked stimulatory effect can be demonstrated by the higher growth rates and activity of bacteria colonizing the rhizosphere than the bulk soil (e.g. Norton and Firestone 1991, Söderberg and Bååth 1998), but there are exceptions of this general rule. Even though plants stimulate microbial activity through the supply of organic substrates, they can at the same time limit microbial growth through depletion of mineral nutrients in the rhizosphere (Bååth et al. 1978, Van Veen et al. 1989, Liljeroth et al. 1990, Parmelee et al. 1993). Experiments done with different grasses have indicated that soil organic matter decomposition, N mineralization, and denitrification can be enhanced in the rhizosphere, but nitrification appears to decrease (Purchase 1974, von Rheinbaben and Trolldenier 1983 and 1984, Wollersheim et al. 1987, Trolldenier 1989, Cheng and Coleman 1990, Wheatley et al. 1990). Plants may thus have dual and counteracting effects on soil microbial populations.

There are not many studies comparing the effects of roots of different tree species, especially boreal ones, on soil microbes. Rates of basal respiration and net N mineralization were higher in the rhizosphere of paper birch (*Betula papyrifera*) than in the rhizospheres of five other tree species (Bradley and Fyles 1995). Microbial communities differed in their use of carboxylic acids and amino acids in the rhizospheres of hybrid larch (*Larix eurolepsis*) and Sitka spruce (*Picea sitchensis*) (Grayston and Campbell 1996). Courtois (1990) compared the fungal flora of the fine roots and rhizospheres of Norway spruce and *Abies alba* and found that tree species had a greater effect on the fungal communities than did the climatic conditions.

As trees affect microbes, microbes, in their turn, affect the trees. The activity of the decomposer population largely determines the nutrient availability to the plant. The whole pool of rhizodeposition is constantly altered by various heterotrophic microbes and their metabolites (Leyval and Berthelin 1993, Meharg and Killham 1995). The presence of microorganisms in the rhizosphere usually increases root exudation (Bolton et al. 1992, Leyval and Berthelin 1993). Microbes can also influence the growth and morphology of roots and the physiology and development of plants by plant hormone production. Different microbes have been found to produce auxin-, cytokinin- and gibberellin-related compounds (e.g. Strzelczyk and Pokojska-Burdziej 1984, Strzelczyk et al. 1985, 1987, Haahtela et al. 1988). Free-living microorganisms can enhance plant growth through the suppression of soil-borne plant pathogenic microbes and deleterious soil microbes (Kloepper 1992). Some microorganisms possess a nitrogenase enzyme, which is capable of reducing atmospheric N to ammonia. This procedure, biological nitrogen fixation, is carried out by either non-symbiotic or symbiotic microorganisms, and has considerable significance for plants (e.g. Killham 1994). As mentioned above, microbes can also have negative effects on plants by competing with them for mineral nutrients. The other negative influence of microbes on plants is the action of root pathogens (Curl 1982).

Boreal forest trees are almost always ectomycorrhizal, and depend on mycorrhizal associations for their nutrient uptake. Mycorrhizas may greatly improve the acquisition of water and nutrients by plant roots, especially the availability of P from sparingly soluble inorganic phosphates (Smith and Read 1997). Mycorrhizas have been found to increase the rate of C translocation into roots (Reid et al. 1983, Leyval and Berthelin 1993), but plants often compensate for this increased C loss by an increase in their photosynthetic rate (Reid et al. 1983, Dosskey et al. 1990, Rousseau and Reid 1990). Mycorrhizas can also change the quality of root exudates, so that mycorrhizal roots produce exudates which are different from those of non-mycorrhizal roots of the same plant (Leyval and Berthelin 1993). The term mycorrhizosphere has been used to describe the enhanced microbial activity in the soil around mycorrhizas as distinguished from that in the rhizosphere soil around nonmycorrhizal roots (Linderman 1988). Mycorrhizas also protect plants from some pathogens (Schenck 1981). In addition to these beneficial effects of mycorrhizas on plant, they can also have harmful effects: mycorrhizal roots of *Pinus radiata* were shown to suppress litter decomposition, in contrast to the effect of non-mycorrhizal roots (Gadgil and Gadgil 1975). Occasionally, mycorrhizal fungi can reduce plant growth, especially during early stages of colonization (Ingham and Molina 1991). It has also been shown that they sometimes may increase the susceptibility of plant roots to infection by pathogenic fungi or nematodes (Schenck 1981).

The infection specificity of ectomycorrhizas varies. Some species infect a wide range of hosts and are termed broad host-range fungi, whereas others are host specific (Smith and Read 1997). Some partner combinations can be better than others for the overall growth of the plant (Mikola 1973). The selection process may also be affected by mycorrhization helper bacteria, which promote the establishment of some mycorrhizal fungi and inhibit others (Garbaye 1994).

Interactions between the plant, the mycorrhizas and the other soil organisms, including bacteria, saprophytic fungi, and soil animals, all of which have not been mentioned here, make the soil and especially the rhizosphere a complicated environment. These biotic interactions can either be positive (mutualistic, associative), neutral, or negative (competitive, predatory), and they vary in nature with plant and fungal species, with microbial and grazer populations, and with abiotic conditions (Ingham and Molina 1991, Beare et al. 1995).

1.4 Studying soil microbes

Soil is a very heterogeneous material, and the determination of soil microbial biomass and microbial activities in soils present many analytical problems, with no standard methods existing. The soil microbial biomass is the primary agent responsible for decomposition processes in soil, and serves both as a source and a sink of nutrients in soil. A majority of soil microbial biomass consists of bacteria and fungi, but microfauna, algeae and viruses are also included. Traditional culture-based methods underestimate the microbial biomass, when compared with microscopic counting, which, however, is tedious (Parkinson and Coleman 1991). During the last few decades several other methods have been developed. The fumigation-incubation method is based on chloroform fumigation of the soil, and subsequent determination of the CO_2 evolving from the decomposing biomass (Jenkinson and Powlson 1976). This method was found to underestimate microbial biomass in acid forest soils (Williams and Sparling 1984, Sparling and Williams 1986, Vance et al. 1987a and 1987b), for which fumigation-extraction is a more suitable method (Vance et al. 1987c). In fumigation-extraction, the elements released from the soil microbes by fumigation, are extracted and measured. The fumigation-extraction method permits not only measurement of microbial biomass C, but also other elements, like N and P (Brookes et al. 1982, Brookes et al. 1985). Not all soil microbes are killed by fumigation. Ingham and Horton (1987) found that protozoan populations were reduced below detection levels by fumigation, but bacterial and fungal populations only to 37-79% of their original populations.

Another commonly used method for determining microbial biomass from soils is substrate-induced respiration, which is thought to measure the active part of microbial biomass in soil. Soil is amended with a readily used substrate, usually glucose, and the following respiratory flush is measured in such a short time that microbes have no time to proliferate (Anderson and Domsch 1978). Microbial respiration without a substrate addition (so called basal respiration), expressed on soil organic C basis, can be used to describe the aerobic mineralization rate of C in soil.

A first step in obtaining a more specific view of the microbial biomass is the separation of bacterial and fungal biomass. The substrate-induced respiration method, supplemented with selective inhibitors, has been used to evaluate bacterial and fungal biomass (Anderson and Domsch 1975, West 1986), but does not work with all soils, especially with ones containing a high concentration of organic matter (Priha, unpublished results). There are also various biomarkers for bacteria and fungi. Muramic acid and diaminopimelic acid (DAP), constituents of the bacterial cell wall peptidoglycan, have been used to determine bacterial biomass (Millar and Casida 1970, Grant and West 1986). Ergosterol is a predominant fungal sterol, and has been used as a measure of fungal biomass (Grant and West 1986), although it has been criticised because the amount of ergosterol varies with fungal species and also within cells of different age (Bermingham et al. 1995). Ergosterol measurement has also been applied to the quantification of ectomycorrhizal fungi, the biomass of which is difficult to measure (Nylund and Wallander 1992). The traditional method of quantifying ectomycorrhizal fungi has been to count mycorrhizal root tips (mycorrhizas), but recognizing them is not always simple. For a more reliable identification of ectomycorrhizas and for classification of different types of ectomycorrhizal fungi, protein based analyses (Rosendahl and Sen 1992) and DNA based methods (Gardes et al. 1991) have been used.

Increasing interest has focused on the microbial community structure. Phospholipids are present in the membranes of virtually all living cells, they are not used as a storage material, and they have a fast turnover rate at least in aquatic environments (Tunlid and White 1992). Different subsets of microbes contain different fatty acids esterified to the phospholipid backbone, or at least different mixtures of them. By analyzing these phospholipid fatty acids (PLFAs) it is possible to study the dynamics of larger groups of organisms by means of specific signature fatty acids. For instance the fatty acid 18:2ω6,9 is typical for fungi, many branched fatty acids are typical for gram-positive bacteria, and monoenoic and cyclopropane fatty acids are typical for gram-negative species (Federle 1986). Another means of studying the community composition of soil microbes is to determine community level physiological profiles (CLPPs) for soil samples with sole-carbon source utilization tests (Biolog) (Garland and Mills 1991). The colour produced from the reduction of tetrazolium violet is used as an indicator of respiration of the carbon sources. As with fatty acids, no individual species can be recognized, but instead the response of the whole microbial, or rather bacterial, community is followed. The Biolog method measures the metabolic abilities of the community, and is thought to reflect the functional potential of the community. The profiling of bacterial communities by denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis (TGGE) of 16S rDNA genes is also on the increase (reviewed by Rosado et al. 1997), although soil, especially ones with a high organic matter content, present problems as a material for molecular studies.

Microbes are mainly responsible for the different reactions involved in the nitrogen cycle in soil. The activities of the nitrogen cycle are usually studied with different incubation experiments. Net ammonification and net nitrification are most often evaluated by incubating soil samples without plant roots for a certain time, and measuring the concentrations of ammonium and nitrate before and after incubation. Fluxes of N can also be measured in field incubations, where less disturbance is caused for the soil, and moisture and temperature conditions fluctuate naturally (Raison et al. 1987). Although having many limitations, the most probable number (MPN) -method is probably the most common method for evaluating the numbers of ammonium- and nitrite-oxidizers in soil (e.g. De Boer et al. 1992, Aarnio and Martikainen 1996, Paavolainen and Smolander 1998). 16S rDNA -based probes have been used in the identification of different genera of ammonium-oxidizers, but these methods are yet not quantitative (Stephen et al. 1996, Kowalchuk et al. 1997).

Denitrification activity can be measured as N_2O production in the presence of acetylene, which blocks nitrous oxide reductase, thus causing the sole end product of denitrification to be N_2O (Yoshinari and Knowles 1976). Denitrification activity or potential measurements allow new enzymes to be synthesized, whereas denitrification enzyme activity (DEA) measurements aim at determining the activity of pre-existing denitrifying enzymes in soil, without allowing denitrifying organisms to proliferate (Luo et al. 1996, Federer and Klemedtsson 1988). The contribution of nitrification on N_2O production can be evaluated by using low partial pressures of acetylene (2.5 - 5 Pa), which inhibit nitrification, but have only a small effect on denitrification (Klemedtsson et al. 1988). Acetylene is also used to evaluate the activity of the N_2-fixing nitrogenase enzyme by the acetylene reduction assay (Hardy et al. 1973).

A more specific picture of the processes of N cycle can be obtained by using ^{15}N (Myrold and Tiedje 1986). ^{15}N can be used as a tracer, which reveals the relative rates and partitioning of added ^{15}N, but it does not provide quantitative estimates of process rates. Isotope dilution experiments involve the addition of ^{15}N into a product pool, and measuring the subsequent dilution of the atom% ^{15}N in this pool permits estimation of short term rates of N processes quantitatively.

2. The aim of the study

The aim of this study was to obtain information about the soil chemistry, microbial biomass, community structure and activities related to C and N cycling in soils under Scots pine, Norway spruce and silver birch. The aim was to determine whether these tree species, and especially their roots, change the soil microbial characteristics.

Microbial biomass C and N, structure of microbial communities, C and N mineralization rates and nitrification and denitrification activities were compared in soils and rhizospheres of pine, spruce and birch.

3. Materials and Methods

3.1 Field sites

Four field sites were studied. Two of them, situated in Karttula and Maalahti, were afforestation experiments established in former agricultural fields (Leikola 1977) (I). The stands were 23-24 years old and both contained three blocks divided into plots of Scots pine (*Pinus sylvestris* L), Norway spruce (*Picea abies* (L.) Karst.) and silver birch (*Betula pendula* Roth) (randomized block design). From both sites soil samples from 0-10 cm layer were studied. From Karttula, also leaf and needle litter samples were collected.

The other two field experiments were forest sites, each containing one plot of each tree species, pine, spruce and birch (II, III). The Punkaharju experiment was established in 1931 and is a fertile site, classified as an *Oxalis acetocella - Vaccinium myrtillus* type (OMT) (Cajander 1949, Beuker 1994). The Uurainen experiment was established in 1936-40, and is less fertile than Punkaharju, classified as a *Vaccinium vitis-idaea* -type (VT) (Cajander 1949, Jaakko Rokkonen, personal communication). At both sites, the soil type was podsol. Soil samples were taken from the humus layer, and 0-3 cm and 3-6 cm mineral soil layers.

3.2 Greenhouse experiments

In the first pot experiment, seeds of pine, spruce and birch were of the Southern Finnish Provenance, and were obtained from the Suonenjoki Research Station of the Finnish Forest Research Institute (IV, V). The seeds were sown into pine, spruce and birch soil from block 2 of the Karttula field afforestation experiment (I).

In the second pot experiment, seedlings of pine, spruce and birch were of the Southern Finnish Provenance, and were obtained from the Suonenjoki Research Station of Finnish Forest Research Institute (VI). The aim was to have seedlings of approximately the same size, instead of the same age, which is why the pine and spruce seedlings were two, and the birch seedlings one-year-old. The seedlings were planted into two different soils, an organic soil and a mineral soil (VI). The soils for this experiment were taken from the pine plot of the less fertile forest site in Uurainen, the organic soil from the organic horizon (humus layer) of the site and the mineral soil from 0-20 cm below the organic horizon (II).

3.3 Microbial determinations

The soil microbial biomass C and N were determined with the fumigation-extraction method (Vance et al. 1987c, Brookes et al. 1985) (I, II, III, IV, VI). Soil microbial biomass C was also measured with the substrate-induced respiration method (Anderson and Domsch 1978, West and Sparling 1986) (I, III, IV, VI). For the rhizosphere samples in paper V, modified versions of these methods, presented by Jensen and Sørensen (1994), were used. The rate of C mineralization was evaluated as CO_2-C production at 14°C (I, III, VI) or 20°C (IV) in 48 h.

Net ammonification and net nitrification were measured by incubating soils at 14°C (I, III, VI) or 20°C (IV) for approximately 6 weeks. Initial NH_4^+-N and (NO_2^-+NO_3^-)-N concentrations, from non-incubated samples, were subtracted from the final (postincubation) NH_4^+-N and (NO_2^-+NO_3^-)-N concentrations. The effect of pH on nitrification was studied with the soil suspension technique described by De Boer et al. (1992) (II). Suspensions, made of humus samples and a mineral nutrient solution containing ammonium, were incubated at room temperature (22°C) for three weeks at either the original pH of the soils, or at pH 6.

The numbers of autotrophic ammonium and nitrite oxidizers were measured by the most-probable number (MPN) method (Martikainen 1985). Inoculated MPN-tubes and control tubes were incubated at room temperature (22°C) for 10 (II, IV) or 14 (VI) weeks. Production of NO_2^- by ammonium oxidizers and NO_3^- by nitrite oxidizers was determined by diphenylamine and Griess-Ilosway reagents, respectively.

The denitrification activity in water saturated soils was measured as N_2O-N production at 14°C (II, VI) or 20°C (IV) in 48 h at 10 kPa partial pressure of acetylene, with (II) or without (II, IV, VI) added KNO_3. For measurement of denitrification enzyme activity (Luo et. al 1996), both glucose and KNO_3 were added, and samples were incubated for 5 h at 20°C (II, V, VI).

For determination of nitrogenase activity, acetylene reduction assay was used. Ethylene release was measured at 14°C (I, II) or 20°C (IV) at ambient air or 10 kPa partial pressure of acetylene. The potential nitrogenase activity was measured by adding glucose.

The ergosterol content of the soils, for determination of the fungal biomass, was measured by using the method of Nylund and Wallander (1992), as modified by Olsson et al. (1996) (IV).

The composition of microbial communities was determined by two methods. The phospholipid fatty acids (PLFAs) were analyzed as described by Frostegård et al. (1993) (III, VI). The sum of PLFAs considered to be mainly of bacterial origin (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 used to represent bacterial biomass (Frostegård and Bååth 1996). The quantity of 18:2ω6,9 was used as an indicator of fungal biomass. Community level physiological profiles (CLPPs) were done according to Campbell et al. (1997) (III, VI). Both Biolog GN type plates, and MT plates with 31 additional carbon sources representing compounds reported in the literature to be plant root exudates, were used.

Plate counts of soils were done on selective media for bacteria and actinomycetes, pseudomonads, and yeasts and fungi (Campbell et al. 1997) (III, VI).

3.4 Soil physical and chemical analyses

The dry matter content of the soils was determined by drying the samples for 18-24 h at 105°C, and the soil organic matter content was measured as loss on ignition from the dried samples at 550°C for 4 h (I-VI). Soil pH was measured in 3:5 (v:v) soil : water (I, II) or soil : 0.01 M $CaCl_2$ (I, IV, VI) suspensions. Total organic C was determined using an automated CHN analyser (I, II). Total soil N was determined with a CHN-analyser (I, II, VI) or by the Kjeldahl method (Halonen et al. 1983) (IV). For determination of other total nutrients (P, K, Ca, Mg), exchangeable nutrients (K, Ca, Mg, Na, Al, Fe, Mn), and soluble P, samples were treated as described by Halonen et al. (1983) and measured with an inductively-coupled plasma emission spectrometer (II). For determination of exchangeable acidity, the samples were extracted with KCl and titrated with NaOH (II).

For characterization of the organic matter, Fourier-transform infrared (FTIR) spectra were run on mortared humus and mineral soil samples (III).

3.5 Analyses of the seedlings

The seedlings were dried at 40°C (IV, VI), or 60°C (V), and shoots and roots were weighed separately. Total N was determined from the needles/leaves by the Kjeldahl method (IV), or with an automated CHN analyzer (VI). Total P of the needles/leaves was measured spectrophotometrically (IV). Root length was measured by scanning with the Mac/WinRHIZO V3.0.2. program (1995) (IV, V). Short root tips were counted using a binocular microscope and classified into those without mycorrhizal infection, developing mycorrhizas, brown sheathed mycorrhizas, *Cenococcum geophilum*, dichotomous and other mycorrhizas (IV).

3.6 Statistical analyses

Means of the measured characteristics between tree species were compared with analysis of variance (Ranta et al. 1989, Milliken and Johnson 1984) (I, IV, V, VI). Significant differences of the means were separated by Tukey's test. The mole percents of the PLFA values and the Biolog values were subjected to principal component analysis (PCA) using a correlation matrix, to see whether the soils group according to the tree species (Mustonen 1995) (III, VI). FTIR spectra were subjected to principal component analysis using a covariance matrix (III).

4. Results and discussion

4.1 Chemical properties of soils under Scots pine, Norway spruce and silver birch

Effects of Scots pine, Norway spruce and silver birch on soil chemical properties were studied both at two field afforestation sites established on former agricultural fields (I), and at two forest sites of different fertility (II, III). At the forest sites, the soil pH(H_2O) varied from 3.8 to 5.0, and was lowest in spruce soil at both sites in all soil layers studied, i.e. the humus layer, and 0-3 cm and 3-6 cm mineral soil layers (II, Table 1). In the humus layer of both sites the pH was about 0.5 units higher under birch than under pine, but in the mineral soil the pH under pine and birch was roughly the same. The organic matter content of the humus layers was variable and was, on average, 48% of d.m. (dry matter), and that of the mineral soil layers 14% and 7% at the OMT- and VT-sites, respectively. There was no consistent influence of tree species. The C:N

Table 1. Soil pH, concentrations of total organic C and total N, and C:N ratios of the soils of the field sites. OMT = *Oxalis acetocella - Vaccinium myrtillus* -type and VT = *Vaccinium vitis-idaea* -type (Cajander 1949).

	pH (H_2O)			Organic C, % of d.m.			Total N, % of d.m.			C : N		
Site	Pine	Spruce	Birch	Pine	Spruce	Birch	Pine	Spruce	Birch	Pine	Spruce	Birch
Karttula field afforestation site	5.7	5.4	5.7	7.9	8.8	10.3	0.42	0.47	0.52	18	18	19
Maalahti field afforestation site	4.7	4.6	4.6	14.3	15.5	14.4	0.88	0.96	0.89	16	16	16
OMT-site humus layer	4.5	4.2	5.0	17.9	25.3	10.7	0.72	0.97	0.48	25	26	22
OMT-site mineral soil layers	4.4	3.8	4.5	6.6	6.9	6.9	0.36	0.32	0.38	18	22	18
VT-site humus layer	4.0	3.8	4.6	33.8	28.6	27.4	0.92	0.90	1.10	37	32	25
VT-site mineral soil layers	4.3	3.9	4.2	3.1	3.6	4.7	0.10	0.11	0.16	30	32	31

d.m. = dry matter

ratios were also variable, ranging from 18 to 37, and in the humus layers of both sites the C:N ratio was lowest under birch. The C:N ratios were lower at the OMT-site compared to the VT-site. At the less fertile VT-site base saturation and concentration of total Ca were highest in birch soil. These results are in accordance with other studies, which have shown that soil pH and base saturation are decreased in spruce soil and increased in birch soil (Nihlgård 1971, Mikola 1985, Miles and Young 1980, Ranger and Nys 1994).

At the field afforestation sites the pH(H_2O) of the soils varied from 4.5 to 5.9, and the organic matter content of the soils from 11 to 48% of d.m. (I, Table 1) The C:N ratios of the soils were between 13 and 21. There were no clear differences, however, in soil chemical characteristics due to tree species.

From the soils of the forest sites, Fourier-transform infrared (FTIR) spectra were run to see whether the soils grouped according to the tree species as regards the characteristics of their organic matter (III). No tree species specific differences were observed, but the sites separated from each other. From heterogeneous materials, such as soil, only some of the major classes of substances and chemical compounds can be identified with FTIR spectroscopy, and only very profound changes can be seen. Ben-Dor and Banin (1995) were able to predict clay content, cation-exchange capacity, carbonate content and organic matter content with near infrared spectra from arid soils in Israel. Haberhauer et al. (1998) compared organic soil layers with FTIR spectroscopy and obtained similar results for all three soils: from the L to H horizon they found a decrease of peak intensity at 1510 cm^{-1}, which correlated to the total C content and C:N ratio of the soil. The different C:N ratios of the soil from OMT- and VT-site could thus influence their separation from each other. It is probable that only more specific groups than those mentioned above can vary due to tree species. Howard et al. (1998) studied two tree species growing in two different soils, and found that four chemical variables of the forty-one studied were significantly influenced by tree species alone. These variables were the content of O in the humic acid, the atomic O to C ratio, the amount of vanillic acid, and the vanillic acid to protocatechuic acid ratio. Such specific changes cannot be revealed with IR-spectroscopy without sample pretreatment.

4.2. Soil microbial biomass and C mineralization rate in stands of different tree species

Microbial characteristics of the soils were affected by tree species at the forest sites. Microbial biomass C and N, and C mineralization rate tended to be lowest under spruce and highest under birch, but the differences varied between sites and in depthwise distribution (II, III). At the more fertile OMT-site microbial biomass C and N were highest under birch and lowest under spruce in all soil layers studied, i.e. in the humus layer and 0-3 cm and 3-6 cm mineral soil layers. C mineralization rate was also highest under birch in all soil layers, but was at the same level under pine and spruce. At the less fertile VT-site microbial biomass C and N, and C mineralization rate were highest under birch in the humus layer, but did not vary in the mineral soil layers, and did not vary between pine and spruce. Microbial biomass C and N often comprised a higher proportion of soil organic C and total soil N under pine and especially under birch than under spruce (Table 2). At both sites, the humus layer was thickest under spruce and thinnest under birch (II), indicating also that decomposition rates in relation to litter production were lowest under spruce and highest under birch. The enhancing effect of birch and decreasing of spruce on decomposition processes has been shown previously (Mikola 1985, Bradley and Fyles 1995). As discussed in the introduction and concluded also by Mikola (1985), probably the more favourable temperature and light conditions at birch stands, and the fact that birch litter is more easily decomposed than that of spruce, partly explain these differences between birch and spruce soils. Scots pine has not been included in the earlier comparisons, and it seemed to be intermediate between birch and spruce regarding effects on soil microbial biomass and C mineralization.

As mentioned above, the stimulating effect of birch depended also on site characteristics, at the more fertile OMT-site it was seen in all soil layers, but at the VT-site only in the humus layer. The effects of above-ground litter are probably more limited to the humus layer, whereas the effects of roots extend deeper. The depthwise distribution of roots may vary at different sites, it is possible that in the mixed soil of OMT-site roots of birch have extended deeper than at the typical podzol profile of the VT-site. Usually the roots of birch extend deeper than those of pine, and especially spruce, whose roots are mostly in the surface soil (Laitakari 1929, 1935). The effect of pine on soil, in comparison with other tree species, depended also on the site: at the OMT-site pine and birch soils had approximately the same microbial activities, but at the VT-site the activities were at the same level in both coniferous soils (II, III).

Table 2. Proportions of microbial biomass C and N of soil organic C and total N, and microbial biomass C:N ratio of the soils at different experiments. FE = fumigation-extraction. For explanation of forest site types, see Table 1.

	FE-derived microbial biomass C, % of total organic C			FE-derived microbial biomass N, % of total N			Microbial biomass C : microbial biomass N		
	Pine	Spruce	Birch	Pine	Spruce	Birch	Pine	Spruce	Birch
Karttula field afforestation site [1]	1.5	1.5	1.5	4.7	4.3	4.7	6	6	6
Maalahti field afforestation site [1]	1.0	1.0	1.0	2.3	2.5	2.6	7	6	6
OMT-site humus layer [1]	2.1	1.8	2.6	6.8	6.6	9.4	8	7	6
OMT-site mineral soil layers [2]	2.1	1.7	2.6	3.0	2.4	4.2	13	17	12
VT-site humus layer [1]	1.5	1.5	2.0	6.9	6.1	7.8	8	8	7
VT-site mineral soil layers [2]	2.2	2.4	2.0	4.4	5.1	4.9	15	15	13
First pot experiment [2]	1.4	1.2	1.6	2.1	1.9	2.5	13	12	12
Second pot experiment, organic soil [1]	0.8	0.8	0.9	3.8	3.6	4.6	7	9	10
Second pot experiment, mineral soil [2]	1.7	1.6	1.8	3.3	3.2	2.8	11	12	14

Conversion factors for microbial biomass C:
[1] $C_{mic} = (1.9 \times FE\text{-}C + 428)\ \mu g\ g^{-1}$ dry matter (Martikainen and Palojärvi 1990)
[2] $C_{mic} = FE\text{-}C / 0.35$ (Sparling et al. 1990)

Conversion factors for microbial biomass N:
[1] $N_{mic} = (1.86 \times FE\text{-}N + 74.82)\ \mu g\ g^{-1}$ d.m. (Martikainen and Palojärvi 1990)
[2] $N_{mic} = FE\text{-}N / 0.54$ (Brookes et al. 1985)

At the field afforestation sites, there was no effect of the different tree species on soil microbial biomass C or C mineralization rate (I). There are probably two reasons why differences were observed at forest sites but not at field afforestation sites: time and previous history of the sites. Probably a long time is needed for trees to cause any changes in bulk soil, and the field afforestation sites were only 23-24 years old, whereas the forest sites were approximately 60 years old. Furthermore, the afforestation sites were untypical due to their agricultural history, which had probably included liming and fertilization of the soil, and caused their pH to be higher and C:N ratio lower than in natural Finnish forest soils (Table 1). The forest sites were typical podzol soils, where trees may exert stronger control on soil microbes. Nevertheless, in the litters from the field afforestation sites, microbial biomass C and C mineralization rate tended to be higher in birch leaf litter than in pine and spruce needles (I). Soil from the field afforestation sites was sampled to the depth of 10 cm and bulked, because there were no clear soil layers to be separated. It may be that there were tree species effects in the surface soil, which was now "diluted" in the larger amount of soil sampled.

Not only trees, but also understorey vegetation had affected soil microbes. At both field afforestation sites and forest sites the understorey vegetation had differentiated under pine, spruce and birch. At the field afforestation sites it contained grasses, herbs and bushes under pine and birch, and either mosses or no ground vegetation with a thick layer of needles under spruce (I). At the forest sites the understorey vegetation consisted of herbs and dwarf shrubs under pine, of mosses and dwarf shrubs under spruce, and of grasses and herbs under birch (II). The decomposition rates of different understorey plants vary considerably, moss litter has a lower pH and decomposes more slowly than the dead parts of most herbs and grasses (Mikola 1954). Especially at field afforestation sites the understorey vegetation under spruce differed so profoundly from that under pine and birch that it probably influenced the comparison of tree species.

4.3 Response of soil N transformations to tree species

Measuring the concentration of inorganic N in soil is a static measure of soil labile N status: it reveals the amount of N available at the moment, but does not reveal anything about the rates of its formation and use. Measuring the net formation of inorganic N in a laboratory incubation describes, in principle, the formation rate of N available for plants. At the field afforestation sites, the concentration of mineral N in soil, or the rate of net formation of mineral N, did not differ statistically significantly between different tree species (I). At the forest sites, different soil layers under birch often contained more mineral N than the corresponding layers under conifers (II). Nevertheless, net formation of mineral N at the forest sites was variable and not clearly affected by the tree species. Often more N was immobilized than produced in the incubation, which was surprising. It is not likely that denitrification had been occurring in the incubation, because the denitrification potential of the majority of soils even at a higher soil moisture was negligible (II). Thus, other explanations for the negative net formation of N in the incubation should be considered. There may have been intensive microbial immobilization in the incubation. It has been shown with ^{15}N studies that net and gross rates of N mineralization and nitrification do not necessarily correlate with each other, and rapid microbial assimilation of N has been suggested to be the main reason (Davidson et al. 1992, Hart et al. 1994, Stark and Hart 1997). Sieving of the soils may have released labile C compounds in the soils, which would have increased microbial biomass and immobilization of N. Even the initial microbial immobilization of N in these soils was relatively high compared to other Finnish forest soils (Martikainen and Palojärvi 1990, Pietikäinen and Fritze 1993, Smolander and Mälkönen 1994), as microbial biomass N was, at its highest, 9% of total soil N.

Another explanation for the discrepancy between mineral N concentration in the field and in the laboratory incubations is that tree roots may have stimulated N mineralization in the field. The roots of trees have been shown both to increase (Fisher and Stone 1969, Bradley and Fyles 1995) and decrease (Parmelee et al. 1993, Bradley and Fyles 1996 and Bradley et al. 1997) N mineralization in soil, and the effect has been shown to vary depending on the soil properties (Parmelee et al. 1993, Bradley and Fyles 1996). In a study by Bradley and Fyles (1995), soils affected by birch (*Betula papyrifera*) seedling root systems mineralized significantly more N than soils under black spruce (*Picea mariana*) and four other tree species. They suggested that high amounts of root labile C compounds in conjunction with rapid mineral-N uptake by birch roots could stimulate microbial communities to acquire nutrients from the native soil (priming effect). Studies with glucose-amended soils, however, suggested that bacteria do not mineralize extra organic N when given a surplus of C (Elliott et al. 1983). Spruce litter, on the other hand, has been shown to depress N availability in soil (Pastor et al. 1987). The differences between N status of soils of different tree species may also vary in forests of different ages: N mineralization was higher in 49-year-old birch and aspen stands compared to white spruce, but at older stands there were no significant differences between the soils of deciduous and coniferous trees (Paré and Bergeron 1996).

Finnish coniferous forest soils generally show negligible net nitrification, unless managed with nitrogen fertilization (Martikainen 1984, Aarnio and Martikainen 1992, Priha and Smolander 1995, Smolander et al. 1995), liming (Priha and Smolander 1995, Smolander et al. 1995), or clear-cutting (Smolander et al. 1998). At the forest sites, pine soils from the OMT-site showed notable nitrification activity in the aerobic incubation of the soils, and so did birch soils, but in only very small amounts (II). Ammonium availability did not seem to be the controller of nitrification, as all the soils initially contained ammonium.

The pH had a significant effect on nitrification activity of the forest soils. This became evident in aerobic soil suspensions with excess ammonium, where microsites with a higher pH cannot be formed. Only nitrifiers from the pine humus layer of the OMT-site produced nitrate at the natural pH of the soil (pH 4.1) (II). When the pH was raised to 6, nitrification started in all soils, although only at a very low rate in soils from the VT-site. The numbers of ammonium and nitrite oxidizers did not differ substantially between tree species in the humus layer of the OMT-site, but at the VT-site ammonium oxidizers were detected only from the birch humus layer. Nitrification potentials and to some extent the nitrification rates have been found to be related to the C:N ratio of the forest floor, with 25-27 being the critical ratio (Paré and Bergeron 1996, Gundersen et al. 1998). The low nitrification activity in the soil suspension experiments and low numbers of nitrifiers at the VT-site can thus be due to the higher C:N ratio, 25-37, compared to the OMT-site, where C:N ratios ranged from 18 to 26. Differences in nitrification activity between different tree species at the OMT-site could not, however, be explained by the C:N ratio. It seemed that different populations of nitrifiers had established under pine, spruce and birch, having different pH demands.

Inhibition of nitrification by allelopathic compounds from plant litter, such as phenolics (Rice and Pancholy 1972, Lodhi and Killingbeck 1980) or terpenoids (White 1986, 1991, Paavolainen et al. 1998) has been suggested to occur especially in climax ecosystems. There are, however, also studies where allelopathy has not been the reason for low or negligible nitrification (Cooper 1986, De Boer and Kester 1996), and some researchers have claimed that the results can be explained with differences in the availability of ammonium (Bremner and McCarty

1988, 1996). Suggestions of allelopathy as an explanation of the differences in nitrification activity in these soils are difficult to make based on these laboratory measurements.

At the field afforestation sites, all soils had a high nitrification activity, as almost all of the produced ammonium had been nitrified in the incubation, and there were no tree species specific differences (I). The high nitrification activity at these sites was probably due to their being former agricultural fields, with a higher pH and a lower C:N ratio than those found in natural Finnish coniferous forest soils, as mentioned earlier.

Heterotrophic nitrification has been shown to be a significant process in forest soils, driven mainly by fungi (reviewed by Killham 1990). In the soils of this study, nitrification was, however, confirmed to be autotrophic, because acetylene completely inhibited it in laboratory incubations (results not shown).

Denitrification in forest soil can be limited by a lack of nitrate, low pH, low moisture and thus high pO_2, low temperature, or lack of a C source (e.g. Federer and Klemedtsson et al. 1988, Willison and Anderson 1991, Henrich and Haselwandter 1991, 1997). Denitrification activity measurements in water saturated soils with and without added nitrate showed that denitrification was mainly limited by lack of nitrate at the forest sites (II). Nevertheless, also other factors played a role, because denitrification activity even with added nitrate was lower in spruce soil than in pine and birch soil at the OMT-site, and very low in all soils of the VT-site. It correlated positively with total organic C and total N, and base saturation of the soil, while denitrifying enzyme activity correlated positively with total N and negatively with C:N ratio. In addition to low nitrification activity, the lower content of total N and higher C:N ratio of the VT-site could influence the lower denitrification activity at the VT-site as compared to the OMT-site.

What proportion of the results from forest sites was caused by aboveground litter and understorey vegetation, and what part by root activities of the trees, cannot be deduced from the field experiments.

4.4 Microbial biomass and C mineralization rate in the rhizospheres

To separate the effects of tree roots from the other effects of the trees, pot experiments were performed. In the first pot experiment seedlings of the same age were studied, as pine, spruce and birch were grown from seeds from one to two growing seasons. In the second pot experiment seedlings of approximately the same size were compared. At the time of harvest there were negligible amounts of dead roots in the pots of both experiments. Therefore, the main influence of roots probably came from their activities, exudation and uptake of water and nutrients. The seedlings had caused no consistent changes in either the soil pH or the concentrations of nutrients in the soils (IV, VI). In the first pot experiment, the soil pH was lowest in plantless soil, but the difference, although statistically significant, was only 0.1 pH-units (IV). In the second pot experiment the soil pH in the organic soil was highest in plantless soil and lowest in birch rhizosphere, but again the differences were very small (VI). In the mineral soil the pH was lowest in spruce rhizosphere and highest in pine rhizosphere and plantless soil.

The results of the pot experiments regarding soil microbial activities had the same trends as the ones from forest sites, indicating that not only microclimate and above-ground litters of these tree species vary, but also their root activities. In the first pot experiment microbial biomass C and N, and C mineralization rate were higher under pine and birch than under spruce and in plantless soils (IV). Microbial biomass under birch also contained a higher proportion of total soil organic C and total N than under spruce and in plantless soil (Table 2). Pine, spruce and birch had, on average, five, one and six meters of roots, respectively. Birch had by far the highest number of root tips, on average 11 450 per seedling, compared to 1900 and 450 in pine and spruce seedlings, respectively. For pine, spruce, and birch, 92, 81 and 76% of these root tips were mycorrhizal. As all of the soil from each pot was analyzed, the amount of roots had a significant effect on the results. This was shown also by the significant correlation between root length, and basal respiration and substrate-induced respiration (IV).

In a more detailed study, rhizosphere soil and "planted bulk soil" were separated from the seedlings (V). The soil adhering to the roots after shaking was defined as rhizosphere soil, and the rest was termed planted bulk soil. Overall, in this study the roots of all three tree species tended to increase microbial biomass C and N, measured by both fumigation-extraction (FE) and substrate-induced respiration (SIR), as compared to unplanted soil, and the increase was higher in the rhizosphere soil than in planted bulk soil. In the rhizospheres the FE-C was at the same level for all the tree species, but SIR was lowest under spruce. In planted bulk soils both FE-C and SIR were lowest under spruce. This suggests that the increasing effect of pine and birch roots on FE-derived microbial biomass C and N, and C mineralization rate was indeed mostly due to their higher amount of roots. In the immediate rhizosphere all tree species had the same effects, but the planted bulk soil was in closer proximity to the roots of pine and birch than those of spruce. The rhizosphere effect is a gradient from roots, and in this study the rhizosphere samples of pine, spruce and birch were comparable, but the planted bulk soil was further in the gradient with spruce than with pine and birch.

The above conclusion was partly supported by the second pot experiment, where seedlings of approximately the same size, and similar layers of soil on the roots, were compared (VI). In the organic soil, C mineralization rate, and in the mineral soil microbial biomass C and N, and C mineralization rate, did not differ in the rhizospheres of pine, spruce and birch.

It is likely that not only the roots of pine and birch extend further than those of spruce, but also the extramatrical mycelium of their mycorrhizas. In both pot experiments, all seedlings were mycorrhizal (IV, V, VI). In the first pot experiment, the amount of ergosterol, an indicator of fungal biomass, was higher under pine and birch, suggesting that they had more extramatrical mycelium in soil than spruce (IV). In the organic soil of the second pot experiment birch rhizosphere contained more of the fungal specific fatty acid $18{:}2\omega6{,}9$ than the others, which could also be due to higher amounts of mycorrhizal mycelium in birch rhizosphere (VI). The extramatrical hyphae of mycorrhizas are included in the FE-C and FE-N measurements. In addition to their direct inclusion in soil microbial biomass, mycorrhizas can also change the soil microbial biomass indirectly by affecting bacterial communities in soil. It has been suggested that the external mycorrhizal mycelium distributes plant C to compartments beyond the rhizosphere (Hobbie 1992), and different ectomycorrhizal fungi have been shown to change bacterial community structure in the mycorrhizosphere (Timonen et al. 1998, Olsson and Wallander 1998).

The rooting densities of pine, spruce and birch in field conditions, especially at soils of the same fertility, are not well known. There are some results, however, indicating that the same differences in rooting densities as found in the pot experiments may occur in the field. Kalela (1949) compared horizontal root systems of spruce and pine of the same size or of the same age, and found that throughout their early growing stages, pine always had larger root systems than spruce. At the age of 110 years and a cubic volume of 0.35 m^3, the trees had root systems of approximately the same size, but after that the root systems of spruce were larger.

Nevertheless, in addition to the amount and extension of roots and mycorrhizas, also the root activities per unit of root differed between tree species, because there were differences in some microbial activities also when the amount of roots did not have an effect on the results. In the first pot experiment SIR was lower in spruce rhizosphere as compared to pine and birch rhizospheres (V), and in the second pot experiment FE-derived microbial biomass C and N were higher in birch rhizosphere than in those of pine and spruce in the organic soil (VI). Not only the quantity, but also the quality of root exudates may differ between species; the root exudates of birch could be better substrates for microbes than those of spruce. Root exudates of sterile seedlings of pine, spruce and birch have been collected, and their chemical characterization is being done (Priha et al., unpublished). Nevertheless, it has to be borne in mind that exudates of mycorrhizal seedlings are bound to be different from sterile ones.

As at the forest sites, where all mechanisms of trees affected the soil, also in the rhizospheres the effects of the tree species on soil microbes were dependent on the soil (VI). In the organic soil substrate-induced and basal respiration did not differ between treatments, including the plantless soil. In the mineral soil, however, both were significantly lower in plantless soil than in the rhizospheres of all tree species. This is in accordance with the results of Parmelee et al. (1993) who found that in the organic soil pine roots and microbes competed with each other for moisture and N, but in the nutrient-poor mineral soil roots provided the main input of substrate, which was more significant than the adverse effect of roots.

4.5 N transformations in the rhizospheres

In all soils of both pot experiments, the concentration of mineral N in soil was highest in pots without plants, probably because of the absence of plant N uptake (IV, VI, Table 3). The concentration of mineral N in soil also varied between different tree species. The differences could largely be explained by differences in plant and microbial N uptake. There was less mineral N under pine and birch than under spruce in the first pot experiment, and correspondingly microbial biomass N was highest under

Table 3. Different pools of N in the pot experiments. For explanation of calculating N_{mic} (microbial biomass N), see Table 2.

		N in needles/ leaves, mg g^{-1} d.m.	N in needles/ leaves, mg	N_{mic}, mg g^{-1} d.m.	Mineral N, mg g^{-1} d.m.	Formation of mineral N, mg g^{-1} d.m. 40 d^{-1}
1st pot experiment	Pine	25	4.1	0.062	0.005	0.042
	Spruce	32	0.9	0.056	0.010	0.031
	Birch	15	2.0	0.074	0.004	0.030
	No seedling			0.050	0.015	0.034
2nd pot experiment, organic soil	Pine	20	61	0.42	0.21	0.16
	Spruce	23	35	0.38	0.35	0.16
	Birch	21	52	0.47	0.04	0.14
	No seedling			0.34	0.47	0.11
2nd pot experiment, mineral soil	Pine	10	23	0.026	0.003	-0.0002
	Spruce	12	20	0.029	0.003	0.0063
	Birch	11	20	0.023	0.003	0.0006
	No seedling			0.025	0.013	0.0012

d.m. = dry matter

them and amount of N in seedlings of pine and birch was higher than in seedlings of spruce (IV, Table 3). In the second pot experiment, the concentration of mineral N was lowest in birch rhizosphere and highest in spruce rhizosphere (VI, Table 3). Correspondingly, microbial biomass N was highest in birch rhizosphere, and the amount of N in needles/leaves of pine and birch higher than in those of spruce. In the mineral soil the amount of N in plants or microbial biomass did not differ between different tree species, and neither did the concentration of N.

There were differences also in the rate of net formation of mineral N between different tree species, but the results differed between the two experiments. In the first pot experiment net formation of mineral N was higher in pine soil than in spruce and birch soil (IV, Table 3). In the second pot experiment net formation of mineral N did not differ between different tree species in the organic soil, but in the mineral soil it was highest in spruce rhizosphere (VI, Table 3). As discussed earlier, in other studies roots of trees have been shown both to increase and decrease N mineralization in soil (Bradley and Fyles 1995, Parmelee et al. 1993). The results from pot experiments did not confirm the idea that birch roots stimulated N mineralization, as suggested on p. 21. The straight effect of roots on the rate of N mineralization, however, could not be assessed. Measuring net formation of N in an incubation reveals whether plants have affected the size or activity of the microbial population in soil. Whether there were differences in the gross N mineralization in the pots, or whether there were differences in microbial N uptake in the laboratory incubations between the tree species, cannot be concluded from these experiments. The use of ^{15}N labelling would give a more accurate means of describing the actual rate of the processes.

In the first pot experiment, where seedlings were growing in soil from the field afforestation site, all soils had a high net nitrification activity, but there was less nitrate under pine and birch than under spruce and in plantless soils (IV). The numbers of both ammonium and nitrite oxidizers were either unaffected or decreased by roots, with the exception of spruce rhizosphere, where the numbers of both were increased (V). There is controversy with regard to effects of plant roots on nitrification. Studies done with some herbaceous plants suggested that nitrification can be suppressed by allelopathy of organic compounds originating from plant roots (Moore and Waid 1971), but also studies showing no such effect exist (Purchase 1974). Probably, most often the availability of ammonium limits nitrification in the rhizosphere, but in this study ammonium concentration did not differ between different tree species. Nitrate can also be lost through denitrification, but denitrification potential was not higher under pine and birch when compared with spruce. Thus, probably pine and birch had been taking up the nitrate produced. Although both non-mycorrhizal and mycorrhizal conifer roots generally prefer ammonium over nitrate as a source of inorganic N (Flaig and Mohr 1992, Marschner et al. 1991, McFee and Stone 1968), Norton and Firestone (1996) showed that mycorrhizal pine roots were more successful competitors with microbes for limited inorganic N when the N source was nitrate vs. ammonium. Microbes, on the other hand, have been shown to compete more effectively for ammonium (Schimel et al. 1989). Either the N limitation in the rhizospheres of pine and birch had caused the use of nitrate or there are differences in the N uptake preferences of pine, spruce, and birch.

Nitrification is generally considered to be a harmful process, because it acidifies the soil and nitrate is easily lost. It is also energetically expensive for plants to assimilate, but on the other hand it can also benefit plants by increasing accessible N. When plants absorb nitrogen as nitrate, the pH in the rhizosphere is raised (Nye 1981), thus counteracting the acidifying effect of the nitrification process. In the first experiment the small increase in the soil pH in pots with seedlings, compared to unplanted pots, may have been caused by uptake of nitrate in pots with seedlings (IV).

In the second pot experiment, seedlings were growing in an organic and a mineral soil taken from the pine plot of the less fertile forest site, which had not shown any detectable nitrification activity (II). In the soils of the pot experiment, net nitrification was detected in the plantless mineral soil (VI). Autotrophic nitrifiers are poor competitors with heterotrophic microbes; in the organic soil and in the rhizospheres of the mineral soil they had probably been outcompeted, but in the plantless mineral soil, where there were less heterotrophic microbes due to lower amount of available C, they were active. Verhagen and Laanbroek (1991, 1992) and Verhagen et al. (1992) showed that heterotrophic *Arthrobacter globiformis* won the competition with *Nitrosomonas europaea* for limiting amounts of ammonium. There were, however, both ammonium and nitrite oxidizers in the rhizospheres of all tree species in the mineral soil. This discrepancy could be due to the fact that when outcompeted, nitrifying bacteria may be able to survive as viable inactive cells which are activated in more favourable conditions, such as MPN media (Verhagen et al. 1992). Although the numbers of ammonium oxidizers have in some studies been shown to reflect reasonably well the changes in potential nitrification activity of soil (Martikainen 1985, Aarnio and Martikainen 1996), there are also studies where the numbers and activities of nitrifying bacteria have had no relationship (Verhagen and Laanbroek 1992, Verhagen et al. 1992). In a study of Berg and Rosswall (1987) a correlation was found between the abundance and activities of ammonium oxidizers, but not of nitrite oxidizers.

In the first pot experiment both N mineralization coefficient (net N mineralization per total soil N; Weier and MacRae 1993) and N efficiency factor (net N mineralization per microbial biomass N; Jenkinson 1988) were lowest under birch (IV). This could indicate a reduced capacity of the microbes in soil affected by birch roots to process N and release it in a form available to plants. It should, however, be borne in mind that some mycorrhizal mycelium probably still remained in the soil despite careful removal of roots, especially in the soils under birch. The mycorrhizal mycelium assimilates N, but does not increase the amount of inorganic N in the soil. This appears to be a problem in N efficiency factor calculations. Although the total amount of N in birch seedlings compared to spruce was high, the concentration of N in the leaves of the birch seedlings was very low (Saarsalmi et al. 1992), and N might have limited the growth of birch. The N concentration in pine and spruce needles, however, indicated a good nutritional status of the seedlings (Jukka 1988, Rikala and Huurinainen 1990). As the measurements in these studies were not planned for evaluation of nutrient budgets, such calculations cannot be made. The different pools of N from the pot experiments, however, can be compared to some extent. As discussed earlier, both in the first pot experiment and in the organic soil of the second pot experiment, pine and birch leaves contained higher amounts of N than those of spruce, and the microbial biomass N was higher (IV, VI, Table 3). Accordingly, the concentration of mineral N in soil was lower under pine and birch. As the demand of N was highest in pine and birch pots, it seems strange that there were no clear differences in the rate of net formation of mineral N between tree species. As discussed earlier, it can be that plant roots had stimulated N mineralization when present. Alternatively plants may have obtained some of the N they need in organic form with the help of ectomycorrhizas (Chalot and Brun 1998, Näsholm et al. 1998). Nevertheless, often under birch, where C is less limiting for microbes, it seems that the competition for N between microbes and the plant is most intensive. Studies for evaluation of the competition for added $^{15}(NH_4)_2SO_4$ between soil microbes and seedlings of pine, spruce and birch have been done, and the analyses of the ^{15}N samples are on the way (Priha et al., unpublished).

In the first pot experiment denitrification activity in water saturated soil was substantial in all soils, but there were no tree species effects, indicating that roots did not influence the denitrification activity (IV). The high activity is likely to be due to the high nitrification activity and thus high availability of nitrate in these soils. In the second pot experiment denitrification activity was low in all soils (VI). As at the forest sites, the availability of substrate seemed to be the main factor controlling denitrification in these soils, because patterns of denitrification followed the concentration of nitrate especially in the organic soils. The activity of pre-existing denitrifying enzymes in soil, however, did not differ substantially between different tree species, as shown by measurements of denitrifying enzyme activity (DEA). On an organic matter basis, there was a higher DEA in the mineral soil than in the organic soil, which could be due to the lower partial pressure of O_2 in the compacting mineral soil as compared to the more aerated organic soil. Potential denitrifying activity in the rhizosphere has been found to correlate with photosynthetic activity (Scaglia et al. 1985) and plant dry weight (Hall et al. 1998). The reason for this has been suggested to be that the metabolic activity of a living plant both reduces the oxygen concentration, and increases the amount of C, by larger amounts for bigger plants with higher photosynthetic activity. In this study there was a positive correlation between DEA and dry weight of all seedlings in the organic soils, but not in the mineral soils (VI).

4.6 The influence of tree species on microbial communities

Not only the size of the microbial biomass, but also the microbial community structure had been influenced by the tree species, as shown by phospholipid fatty acids (PLFAs) being grouped according to tree species at the two different forest sites (III). Spruce and birch differed most clearly from each other both in humus layer and mineral soil layer, whereas pine was close to spruce in the humus layer, and close to birch in the mineral soil layer. PLFAs 18:1ω7 and 16:1ω7c, common in gram-negative bacteria (Haack et al. 1994), and PLFA 16:1ω5, present in bacteria (Nichols et al. 1986), and in arbuscular mycorrhizal fungi (Olsson et al. 1995), were relatively more abundant in birch and pine soils compared to spruce. PLFA 20:4, which is found in eucaryotic organisms (Federle 1986) was common in birch soil from the OMT-site. The presence of 16:1ω5 could be due to the abundance of grasses in birch and pine plots, which can have arbuscular mycorrhizas. PLFA 10Me18:0, typical for actinomycetes (Kroppenstedt 1985), increased in the humus layer under birch, but in mineral soil did not differ under different tree species. The higher pH in the birch soil at the OMT-site (II), could be one reason for this, since actinomycetes are known to have a higher pH optimum than other soil bacteria or fungi (Killham 1994). Besides, the density of *Frankia* has been shown to be high in some birch soils (Smolander 1990).

The relative amounts of PLFAs 16:1ω7t and anteiso-branched a17:0 were higher in spruce soil, and the amounts of branched i16:1 and 10Me16:0, which are typical for gram-positive bacteria (O'Leary and Wilkinson 1988) were higher in spruce and pine soils compared to birch (III). Branched fatty acids have previously been found to increase as a result of simulated acid rain leading to a decrease in soil pH (Pennanen et al. 1998), which is in accordance with the results of this study, as spruce plots had the lowest pH and birch plots the highest (II). An increased ratio of 16:1ω7t to 16:1ω7c in spruce soil could be due to stress in spruce soil, because an increase in the ratio of *trans/cis* PLFAs has been suggested to indicate starvation (Guckert et al. 1986) or desiccation

(Kieft et al. 1994) in a bacterial community. This is consistent with the lower microbial biomass and C mineralization rate under spruce. Nevertheless, the connection was not as straightforward as this, because in the mineral soil of the VT-site spruce did not differ from pine and spruce, and C mineralization rate did not differ between pine and spruce.

The changes in PLFA composition due to birch and spruce were largely in accordance with the ones of Saetre (1998) and Saetre and Bååth (personal communication). They compared PLFA patterns in soils of Norway spruce and downy birch both in laboratory and field experiments, and found that PLFAs 16:1ω7c, 16:1ω5, 18:1ω7 and 18:1ω9c increased with birch influence and PLFAs 20:0, a17:0, 10Me17:0 and br18:0 increased with spruce influence. They concluded that the changes in microbial communities were connected to differences in the quality of organic matter associated with the two tree species.

The ratio of fungal to bacterial PLFAs did not differ between different tree species, but was almost twice higher at the less fertile VT-site compared to the fertile OMT-site (III). This is in accordance with Pennanen et al. (submitted), who found that the relative proportion of fungi decreased along a fertility gradient from less productive sites to nutrient-rich sites.

There were shifts in the microbial community structure in the rhizospheres of pine, spruce and birch seedlings in the organic soil, but not in the mineral soil (VI). In the organic soil, the fatty acids more common in birch rhizosphere than in pine and spruce rhizosphere and especially plantless soil, were the fungal specific 18:2ω6,9 and many branched fatty acids, which have commonly been found in gram-positive bacteria (O'Leary and Wilkinson 1988). The increasing amount of 18:2ω6,9, and the increased ratio of fungal to bacterial PLFAs from plantless soil to birch rhizosphere was possibly caused by mycorrhizal fungi instead of saprophytes. The PLFA pattern of the pine rhizosphere in the organic soil separated slightly from the PLFA patterns of spruce rhizosphere and the unplanted soil, but the changes in the individual PLFAs could not be clearly associated to certain groups of bacteria. The PLFA patterns of the spruce rhizosphere and the unplanted soil were relatively similar. The PLFAs more common in them were mostly monounsaturated, typical to gram-negative bacteria (Wilkinson 1988), even though the abundance of a15:0, common in gram-positive bacteria, was also high. The relative amount of bacterial PLFA 16:1ω5 (Nichols et al. 1986) was highest in the unplanted organic soil, and also higher in spruce rhizosphere than in the rhizospheres of pine and birch. In the study of Frostegård et al. (1996), 16:1ω5 decreased during incubation, and they suggested that this PLFA may reflect the dynamics of organisms that are responding to changes in the C status of the soils. It could be that pine and birch had been taking up more nutrients than the smaller spruce seedlings, which had caused more competition between microbes and plants and also a less favourable C status of the soil towards the end of the growing season.

The changes in PLFA composition due to tree species were not similar at the field sites and in the rhizospheres (III, VI). It is possible that at the field sites the litter of the trees and the understorey vegetation had exerted the strongest control on the microbial communities, but in the rhizospheres the composition of root exudates had the main influence. In addition, the chemical characteristics of the soils also differed at field sites and in the rhizospheres of these tree species: at the field sites soil pH was higher under pine and especially birch than under spruce, but in the rhizospheres this was not the case.

The separation of tree species at field sites by CLPPs was not clear, and replicate soil samples varied greatly in the rate and extent of their substrate use (III). Different substrate combinations did not differ in their separation power, even though Campbell et al. (1997) obtained a more distinctive discrimination of microbial communities of different grassland sites using the 61 exudate sources than all 125 C sources. Bååth et al. (1998) suggested that the Biolog method probably work better in environments like the rhizosphere, where a larger proportion of the community is active compared to bulk soil.

In the rhizospheres the CLPPs differentiated birch rhizosphere from pine and spruce rhizospheres and plantless organic soil (VI). The C sources from the MT-plate had a tendency of separating also pine and spruce rhizosphere from the unplanted soil. There were negligible amounts of colony-forming pseudomonads in the birch rhizosphere in the organic soil, and also the average well colour development was much lower than in the other soils. This could influence the strong separation of birch in CLPPs, as *Pseudomonas* species have been found to be enriched in Biolog wells (Grayston et al. 1998). This is in accordance with the PLFA results, which showed a high amount of gram-positive bacteria in birch rhizosphere. The low number of *Pseudomonas* species in birch rhizosphere was surprising, given that they are usually increased in rhizospheres (reviewed by Bolton et al. 1992). Nevertheless, fluorescent pseudomonads were commonly isolated from Scots pine mycorrhizospheres in nursery peat, but they were nearly absent from outer mycorrhizospheres in pine forest humus, where *Bacillus* species were more important (Timonen et al. 1998). Because birch roots filled the pots almost totally, birch rhizosphere samples probably contained more soil around the external hyphae of mycorrhizas than pine and spruce samples. Thus, there might have been a higher dominance of *Bacillus* species in birch rhizosphere samples compared to those of pine and spruce.

There has been a lot of discussion regarding the reliability of the methods for measuring microbial community structure and function and indeed what they actually measure. In both field samples and rhizosphere samples PLFAs grouped the samples more clearly than CLPPs did. Other studies have also shown that PLFAs can be more sensitive in detecting shifts in microbial community structure than Biolog (Buyer and Drinkwater 1997, Fritze et al. 1997, Bååth et al. 1998, Pennanen et al. 1998). This could either mean that the microbial communities change their structure without changing their functions, or that the Biolog method is less sensitive than the PLFAs. The latter case is probably true, as it has recently been suggested that Biolog measures the structural rather than functional properties, because it measures potential, and not the actual substrate use of the community (Garland et al. 1997, Bååth et al. 1998). As such, the Biolog method could be more limited than PLFAs, because Biolog only measures the metabolic profiles of culturable bacteria, whereas PLFAs assess the whole community.

It is plausible that the use of ecologically relevant C sources, such that can be found from the soils, would give the best separation in Biolog. Campbell et al. (1997) found the separation of microbial communities to be more distinct using 61 exudate C sources from the GN- and MT-plates than using the 125 C sources in GN- and MT-plates together. In this study, however, this was not the case, not in the field soils, nor in the rhizosphere soils (III, VI). The C sources in the MT-plate alone had a tendency of separating in addition to birch, also pine and spruce rhizosphere from the unplanted soil. This might indicate that the substrates in the MT-plates were more ecologically relevant for these soils than the ones in GN-plates. Notable was the preferential use of many phenolic compounds in birch rhizosphere.

As mentioned above, there were shifts in the microbial communities in the rhizospheres of pine, spruce and birch growing in organic soil, but not in mineral soil (VI). The reason for this could be that in the organic soil there was more diversity to start with, which makes it possible that different groups are enriched in different conditions, whereas the original microbial community in the mineral soil probably was less diverse. In addition, it is not only the roots of the seedlings that affect the microbial communities in soil, but also different mycorrhizal species and their exudates have been found to change the soil bacterial communities (Timonen et al. 1998, Olsson and Wallander 1998). We did not determine how many and what kind of mycorrhizal infections the seedlings had in this study, but it could be that the seedlings were more mycorrhizal in the organic soil, which would have caused the mycorrhizal effect to be stronger. It has been suggested that the conditions for mycorrhiza formation are better in organic than in mineral soil (Meyer 1974, Harvey et al. 1976), but the opposite has also been found (Alvarez et al. 1979).

4.7 Concluding remarks

In summary, several soil properties differed under Scots pine, Norway spruce and silver birch at the approximately 60-year-old forest sites (Tables 1 & 4). Soil pH, microbial biomass C and N, and C mineralization rate tended to be highest in birch soil and lowest in spruce soil, but the effects varied between sites and in depthwise distribution. Not only the size of the microbial biomass, but also the microbial community structure had changed under different tree species. This was shown by differences in nitrifying and denitrifying populations, and in phospholipid fatty acid profiles. At 23-24-year old afforestation sites in fields formerly used for agriculture there were, however, no tree species specific changes in soil microbial biomass and activities, even though in the litters of pine, spruce and birch microbial biomass and activity did vary. Probably a long time is needed for trees to cause any changes in bulk soil, and the changes also depend on the original soil type.

The same trends as at the forest sites were found also in pot experiments, where only the roots of seedlings influenced soil microbes: microbial biomass C and N, and C mineralization rate were higher under pine and birch than under spruce and in plantless soils. The stimulating effect of pine and especially birch roots on soil microbes seemed to be mostly due to their higher amount of roots and root tips, and of their roots and mycorrhizas extending further than those of spruce, thus providing more exudates. Nevertheless, there were also qualitative differences in the effects of roots, because differences in microbial biomass and activities

Table 4. A generalized overall picture of the effects of tree species on soil microbes and their activities. The symbols can only be compared within one experiment. C_{mic} (microbial biomass C), C mineralization and N_{mic} (microbial biomass N): +++ > ++ > +. Net formation of mineral N and net nitrification: + = mineralization, 0 = no net effect, - = immobilization into microbes. PLFAs (phospholipid fatty acids) and CLPPs (community level physiological profiles; Biolog): different symbols indicate varying patterns. * = results could not be simplified into this form.
P = pine, S = spruce and B = birch. For explanation of forest site types, see Table 1.

		Paper	C_{mic}			C mineralization			N_{mic}			Net formation of mineral N			Net nitrification			PLFAs			CLPPs		
			P	S	B	P	S	B	P	S	B	P	S	B	P	S	B	P	S	B	P	S	B
Field experiments	Field afforest. sites	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
	OMT humus layer	II, III	++	+	++	+	+	++	++	+	+++	+	++	-	++	0	0/+	✧	✦	o		*	
	OMT mineral soil	II, III	++	+	+++	+	+	++	++	+	+++	+	+	0	++	0	0/+	✧	✦	✧		*	
	VT humus layer	II, III	+	+	++	+	+	++	+	+	++	-	0	+	0	0	0	✧	✦	o		*	
	VT mineral soil	II, III	+	+	+	+	+	+	+	+	+	0/-	-	0	0	0	0	✧	✦	✧		*	
Pot experiments	1st	IV	++	+	+++	++	+	++	++	+	+++	++	+	+	++	+	+						
	2nd, organic soil	VI	+	+	++	+	+	+	+	+	++	+	+	+	0	0	0	✧	✦	o	✧	✧	✦
	2nd, mineral soil	VI	+	+	+	+	+	+	+	+	+	0	++	+	0	0	0	✧	✧	✧	✧	✧	✧

were observed also when the amount of roots did not have an effect. In addition, the microbial community structure had changed in the rhizospheres of different tree species.

In the rhizosphere of birch, where C was less limiting for microbes, the competition for N between microbes and plants was most intensive. There were differences also in the rate of net formation of mineral N between tree species, but the results were different for different experiments.

The effects of roots of trees also depended on the soil type. In an organic soil there were differences in microbial biomass and microbial community structure between rhizospheres of different tree species. In the mineral soil, however, the roots of all tree species stimulated C mineralization compared to plantless soil, and did not affect microbial biomass or microbial community structure.

In conclusion, soil chemical and microbial characteristics often differed in soils from stands of Scots pine, Norway spruce and silver birch, but not at all stands. Microbial biomass and activity was often higher under pine and especially birch than under spruce. The roots of these tree species alone also affected microbes, and the tree species specific changes, if any, tended to be similar as in the field.

5. Summary

Different tree species tend to establish in different soils, but trees also themselves change the soil underneath. The effects of Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) Karst.) and silver birch (*Betula pendula* Roth) on soil microbial and chemical properties were studied. Two forest sites of different fertility and two afforestation sites on former agricultural fields were included. The effects of roots of trees were separated in greenhouse experiments having either seedlings of the same age or of approximately the same size.

Soil chemistry and microbial activities differed in soils of pine, spruce and birch at the forest sites, which were approximately 60 years old. Soil pH, microbial biomass C and N, and C mineralization rate tended to be highest in birch soil and lowest in spruce soil. At the more fertile site these changes were seen both in the humus layer and in the mineral soil layers, but at the less fertile site it was only seen in the humus layer. Also

activities of nitrifying and denitrifying bacteria, and microbial community structure had changed under different tree species. At the 23-24-year-old field afforestation sites, however, there were no tree species specific changes in microbial biomass or activities.

In pot experiments, where only the roots of the seedlings affected microbes, microbial biomass C and N, and C mineralization rate were higher under pine and especially birch than under spruce and in plantless soils. The stimulating effect of pine and especially birch roots on soil microbes seemed to be mostly due to their higher amount of roots and root tips and of their roots and mycorrhizas extending further than those of spruce, thus providing more exudates for soil microbes. Nevertheless, there were also qualitative differences in the effects of roots, because differences in microbial biomass, community structure, and activities were observed also when the amount of roots did not have an effect. In the rhizosphere of birch, where C was less limiting for microbes, the competition for N between microbes and plants was most intensive. As in the field, the effects of roots of trees also depended on the soil type. In the organic soil there were differences in microbial biomass and microbial community structure between the rhizospheres of different tree species. In the mineral soil, however, roots of all tree species stimulated C mineralization compared to the plantless soil, and did not affect microbial biomass or microbial community structure.

References

Aaltonen VT (1941) Metsämaamme valtakunnan metsien toisen arvioinnin tulosten valossa. Zusammenfassung: Die finnischen Waldböden nach den Erhebungen der zweiten Reichswaldschätzung. Communicationes Instituti Forestalis Fenniae 29(5), 62 p.

Aarnio T & Martikainen PJ (1992) Nitrification in forest soil after refertilization with urea or urea and dicyandiamide. Soil Biology and Biochemistry 24: 951-954

Aarnio T & Martikainen PJ (1996) Mineralization of carbon and nitrogen, and nitrification in Scots pine forest soil treated with fast- and slow-release nitrogen fertilizers. Biology and Fertility of Soils 22: 214-220

Alban DH (1982) Effects of nutrient accumulation by aspen, spruce, and pine on soil properties. Soil Science Society of America Journal 46: 853-861

Alvarez IF, Rowney DL & Cobb FW Jr (1979) Mycorrhizae and growth of white fir seedlings in mineral soil with and without organic layers in a California forest. Canadian Journal of Forest Research 9: 311-315

Anderson JPE & Domsch KH (1975) Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Canadian Journal of Microbiology 21: 314-322

Anderson JPE & Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10: 215-221

Bååth E, Lohm U, Lundgren B, Rosswall T, Söderström B, Sohlenius B & Wirén A (1978) The effect of nitrogen and carbon supply on the development of soil organism populations and pine seedlings: a microcosm experiment. OIKOS 31: 153-163

Bååth E, Díaz-Raviña M, Frostegård Å & Campbell CD (1998) Effect of metal-rich sludge amendments on the soil microbial community. Applied and Environmental Microbiology 64: 238-245

Barford C & Lajhta K (1992) Nitrification and nitrate reductase activity along a secondary successional gradient. Plant and Soil 145: 1-10

Bauhus J, Paré D & Côté L (1998) Effects of tree species, stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. Soil Biology and Biochemistry 30: 1077-1989

Beare MH, Coleman DC, Crossley DA Jr, Hendrix PF & Odum EP (1995) A hierarchical approach to evaluating the significance of soil biodiversity to biogeochemical cycling. Plant and Soil 170: 5-22

Ben-Dor E & Banin A (1995) Near-infrared analysis as a rapid method to simultaneously evaluate several soil properties. Soil Science Society of America Journal 59: 364-372

Berg B (1984) Decomposition of root litter and some factors regulating the process: long-term root litter decomposition in a Scots pine forest. Soil Biology and Biochemistry 16: 609-617

Berg B (1986) Nutrient release from litter and humus in coniferous forest soils: a mini review. Scandinavian Journal of Forest Research 1: 359-369

Berg B & Rosswall T (1987) Seasonal variations in abundance and activity of nitrifiers in four arable cropping systems. Microbial Ecology 13: 75-87

Berg B & Wessén B (1984) Changes in organic-chemical components and ingrowth of fungal mycelium in decomposing birch leaf litter as compared to pine needles. Pedobiologia 26: 285-298

Bermingham S, Maltby L & Cooke RC (1995) A critical assessment of the validity of ergosterol as an indicator of fungal biomass. Mycological Research 99: 479-484

Beuker E (1994) Long-term effects of temperature on the wood production of *Pinus sylvestris* L. and *Picea abies* (L.) Karst. in old provenance experiments. Scandinavian Journal of Forest Research 9: 34-45.

Binkley D (1995) The influence of tree species on forest soils: processes and patterns. In: Proceedings of the Trees and Soil Workshop, Lincoln University 28 February - 2 March 1994. Edited by: Mead DJ & Cornforth IS. Agronomy Society of New Zealand Special Publication 10, Lincoln University Press, Canterbury. p. 1-33

Binkley D & Valentine D (1991) Fifty-year biogeochemical effects of green ash, white pine and Norway spruce in a replicated experiment. Forest Ecology and Management 40: 13-25

Bolton H Jr, Fredrickson JK & Elliott LF (1992) Microbial ecology of the rhizosphere. In: Soil Microbial Ecology, Applications in Agricultural and Environmental Management. Edited by: Metting FB Jr. Marcel Dekker Inc, New York. p. 27-63

Bowen GD & Rovira AD (1991) The rhizosphere, the hidden half of the hidden half. In: Plant Roots - The Hidden Half. Edited by: Waisel Y, Eshel A & Kafkafi U. Marcel-Dekker, New York. p. 641-649

Bradley RL & Fyles JW (1995) Growth of paper birch (*Betula papyrifera*) seedlings increases soil available C and microbial acquisition of soil-nutrients. Soil Biology and Biochemistry 27: 1565-1571.

Bradley RL & Fyles JW (1996) Interactions between tree seedling roots and humus forms in the control of soil C and N cycling. Biology and Fertility of Soils 23: 70-79

Bradley RL, Titus BD & Fyles JW (1997) Nitrogen acquisition and competitive ability of *Kalmia angustifolia* L., paper birch (*Betula papyrifera* Marsh.) and black spruce (*Picea mariana* (Mill.) B.S.P.) seedlings grown on different humus forms. Plant and Soil 195: 209-220

Bremner JM & McCarty GW (1988) Effects of terpenoids on nitrification in soil. Soil Science Society of America Journal 52: 1630-1633

Bremner JM & McCarty GW (1996) Inhibition of nitrification in soil by allelochemicals derived from plants and plant residues. Soil Biochemistry 8: 181-218

Brookes PC, Powlson DS & Jenkinson DS (1982) Measurement of microbial biomass phosphorus in soil. Soil Biology and Biochemistry 14: 319-329

Brookes PC, Landman A, Pruden G & Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17: 837-842

Buyer JS & Drinkwater LE (1997) Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. Journal of Microbiological Methods 30: 3-11

Cajander AK (1949) Forest types and their significance. Acta Forestalia Fennica 56(5), 71 p.

Campbell CD, Grayston SJ & Hirst DJ (1997) Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. Journal of Microbiological Methods 30: 33-41

Chalot M & Brun A (1998) Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. FEMS Microbiology Reviews 22: 21-44

Chang SX, Weetman GF & Preston CM (1996) Understory competition effect on tree growth and biomass allocation on a coastal old-growth forest cutover site in British Columbia. Forest Ecology and Management 83: 1-11

Chapman K, Whittaker JB & Heal OW (1988) Metabolic and faunal activity in litters of tree mixtures compared with pure stands. Agriculture, Ecosystems and Environment 24: 33-40

Cheng W & Coleman DC (1990) Effect of living roots on soil organic matter decomposition. Soil Biology and Biochemistry 22: 781-787

Cooper AB (1986) Suppression of nitrate formation within an exotic conifer plantation. Plant and Soil 93: 383-394

Courtois H (1990) Endophytische Mikropilze in Fichtenfeinwurzeln. Summary: Endophytic microfungi in fine roots of Norway spruce. Allgemeine Forst und Jagdzeitung 161: 189-198

Curl EA (1982) The rhizosphere: relation to pathogen behavior and root disease. Plant Disease 66: 624-630

Davidson EA, Hart SC & Firestone MK (1992) Internal cycling of nitrate in soils of a mature coniferous forest. Ecology 73: 1148-1156

De Boer W, Tietema A, Klein Gunnewiek PJA & Laanbroek HJ (1992) The chemolithotrophic ammonium-oxidizing community in a nitrogen-saturated acid forest soil in relation to pH-dependent nitrifying activity. Soil Biology and Biochemistry 24: 229-234

De Boer W & Kester RA (1996) Variability of nitrification potentials in patches of undergrowth vegetation in primary Scots pine stands. Forest Ecology and Management 86: 97-103

Dosskey MG, Linderman RG & Boersma L (1990) Carbon-sink stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizas. New Phytologist 115: 269-274

Elliott ET, Cole CV, Fairbanks BC, Woods LE, Bryant RJ & Coleman DC (1983) Short-term bacterial growth, nutrient uptake, and ATP turnover in sterilized, inoculated and C-amended soil: the influence of N availability. Soil Biology and Biochemistry 15: 85-91

Eyre SR (1968) Vegetation and soils: A world picture. Edward Arnold, Bath, UK. 328 p.

Federer CA & Klemedtsson L (1988) Some factors limiting denitrification in slurries of acid forest soils. Scandinavian Journal of Forest Research 3: 425-435

Federle TW (1986) Microbial distribution in soil - new techniques. In: Perspectives in Microbial Ecology: Proceedings of the Fourth International Symposium on Microbial Ecology, Ljubljana 24-29 August 1986. Edited by: Megusar F & Gantar M. Slovene Society for Microbiology, Ljubljana. p. 493-498

Finer L, Messier C & De Grandpe L (1997) Fine-root dynamics in mixed boreal conifer - broad-leafed forest stands at different successional stages after fire. Canadian Journal of Forest Research 27: 304-314

Finzi AC & Canham CD (1998) Non-additive effects of litter mixtures on net N mineralization in a southern New England forest. Forest Ecology and Management 105: 129-136

Fisher RF & Stone EL (1969) Increased availability of nitrogen and phosphorus in the root zone of conifers. Soil Science Society of America Proceedings 33: 955-961

Flaig H & Mohr H (1992) Assimilation of nitrate and ammonium by the Scots pine (*Pinus sylvestris*) seedling under conditions of high nitrogen supply. Physiologia Plantarum 84: 568-576

Fritze H, Pennanen T & Vanhala P (1997) Impact of fertilizers on the humus layer microbial community of Scots pine stands growing along a gradient of heavy metal pollution. In: Microbial communities, functional versus structural approaches. Edited by: Insam H & Rangger A. Springer-Verlag, Berlin. p. 68-83

Frostegård Å & Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22: 59-65

Frostegård Å, Bååth E & Tunlid A (1993) Shifts in the structure of soil microbial communities in limed forest as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25: 723-730

Frostegård Å, Tunlid A & Bååth E (1996) Changes in microbial community structure during long-term incubation in two soils experimentally contaminated with metals. Soil Biology and Biochemistry 28: 55-63

Gadgil RL & Gadgil PD (1975) Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata*. NZ Journal of Forestry Science 5: 33-41

Gallet C & Lebreton P (1995) Evolution of phenolic patterns in plants and associated litters and humus of a mountain forest ecosystem. Soil Biology and Biochemistry 27: 157-165

Garbaye J (1994) Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytologist 128: 197-210
Gardes M, White TJ, Fortin JA, Bruns TD and Taylor JW (1991) Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochodrial ribosomal DNA. Canadian Journal of Botany 69: 180-190
Gardiner AS (1968) The reputation of birch for soil improvement. Forestry Commission Research and Development Paper 67, 9 p.
Garland JL & Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Applied and Environmental Microbiology 57: 2351-2359
Garland JL, Cook KL, Loader CA & Hungate BA (1997) The influence of microbial community structure and function on community-level physiological profiles. In: Microbial communities, functional versus structural approaches. Edited by: Insam E & Ranger A. Springer-Verlag Berlin. p. 171-183
Grant WD & West AW (1986) Measurement of ergosterol, diaminopimelic acid and glucosamine in soil: evaluation as indicators of microbial biomass. Journal of Microbiological Methods 6: 47-53
Grayston SJ & Campbell CD (1996) Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (*Larix eurolepsis*) and Sitka spruce (*Picea sitchensis*). Tree Physiology 16: 1031-1038
Grayston SJ, Vaughan D & Jones D (1996) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5: 29-56
Grayston SJ, Wang S, Campbell CD & Edwards AC (1998) Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry 30: 369-378
Guckert JB, Hood MA & White DC (1986) Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: Increases in the *trans/cis* ratio and proportions of cyclopropyl fatty acids. Applied and Environmental Microbiology 52, 794-801
Gundersen P, Emmett BA, Kjønaas OJ, Koopmans CJ & Tietema A (1998) Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. Forest Ecology and Management 101: 37-55
Haahtela K, Laakso T, Nurmiaho-Lassila E-L, Rönkkö R & Korhonen TK (1988) Interactions between N_2-fixing enteric bacteria and grasses. Symbiosis 6: 139-150
Haack SK, Garchow H, Odelson DA, Forney LJ & Klug MJ (1994) Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. Applied and Environmental Microbiology 60: 2483-2493
Haberhauer G, Rafferty B, Strebl F & Gerzabek MH (1998) Comparison of the composition of forest soil litter derived from three different sites at various decompositional stages using FTIR spectroscopy. Geoderma 83: 331-342
Haimi J & Huhta V (1990) Effects of earthworms on decomposition processes in raw humus forest soil: A microcosm study. Biology and Fertility of Soils 10: 178-183
Haimi J, Huhta V & Boucelham M (1992) Growth increase of birch seedlings under the influence of earthworms - a laboratory study. Soil Biology and Biochemistry 24: 1525-1528

Hall JM, Paterson E & Killham K (1998) The effect of elevated CO_2 concentration and soil pH on the relationship between plant growth and rhizosphere denitrification potential. Global Change Biology 4: 209-216

Halonen O, Tulkki H & Derome J (1983) Nutrient analysis methods. Metsäntutkimuslaitoksen tiedonantoja 121, 28 p.

Hardy RWF, Burns RC & Holsten RD (1973) Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biology and Biochemistry 5: 47-81

Harris MM & Safford LO (1996) Effect of season and four tree species on soluble carbon content in fresh and decomposing litter of temperate forests. Soil Science 161: 130-135

Hart SC, Nason GE, Myrold DD & Perry DA (1994) Dynamics of gross nitrogen transformations in an old-growth forest: the carbon connection. Ecology 75: 880-891

Harvey AE, Larsen MJ & Jurgensen MF (1976) Distribution of ectomycorrhizae in a mature Douglas-fir / larch forest soil in western Montana. Forest Science 22: 393-398

Helal HM & Sauerbeck DR (1983) Method to study turnover processes in soil layers of different proximity to roots. Soil Biology and Biochemistry 15: 223-225

Helal HM & Sauerbeck D (1986) Effect of plant roots on carbon metabolism of soil microbial biomass. Zeitschrift für Plfanzenernaehrung und Bodenkunde 149: 181-188

Henrich M & Haselwandter K (1991) Denitrifying potential and enzyme activity in a Norway spruce forest. Forest Ecology and Management 44: 63-68

Henrich M & Haselwandter K (1997) Denitrification and gaseous nitrogen losses from an acid spruce forest soil. Soil Biology and Biochemistry 29: 1529-1537

Hobbie SE (1992) Effects of plant species on nutrient cycling. TREE 7: 336-339

Howard PJA, Howard DM & Lowe LE (1998) Effects of tree species and soil physicochemical conditions on the nature of soil organic matter. Soil Biology and Biochemistry 30: 285-297

Huhta V (1979) Effects of liming and deciduous litter on earthworm (*Lumbricidae*) populations of a spruce forest, with an inoculation experiment on *Allolobophora caliginosa*. Pedobiologia 19: 340-345

Hyvärinen A (1990) Deposition on forest soils - effect of tree canopy on throughfall. In: Acidification in Finland. Edited by: Kauppi P, Anttila P & Kenttämies K. Springer-Verlag, Berlin. p. 199-213

Ingham ER & Horton KA (1987) Bacterial, fungal and protozoan responses to chloroform fumigation in stored soil. Soil Biology and Biochemistry 19: 545-550

Ingham ER & Molina R (1991) Interactions among mycorrhizal fungi, rhizosphere organisms, and plants. In: Microbial mediation of plant - herbivore interactions. Edited by: Barbosa P, Krischik VA & Jones CG. John Wiley & Sons, New York. p. 169-197

Jenkinson DS (1988) Determination of microbial biomass carbon and nitrogen in soil. In: Advances in nitrogen cycling in agricultural ecosystems. Edited by: Wilson JR. CAB International, Wallingford, U.K. p. 368-386

Jenkinson DS & Powlson DS (1976) The effects of biocidal treatments on metabolism in soil - V. A method for measuring soil biomass. Soil Biology and Biochemistry 8: 209-213

Jensen LS & Sørensen J (1994) Microscale fumigation-extraction and substrate-induced respiration methods for measuring microbial biomass in barley rhizosphere. Plant and Soil 162: 151-161

Johansson M-B (1995) The chemical composition of needle and leaf litter from Scots pine, Norway spruce and white birch in Scandinavian forests. Forestry 68: 49-62

Jukka L (ed.) (1988) Metsänterveysopas. Metsätuhot ja niiden torjunta. (Forest health guide. Forest damaging agents and their control). Samerka Oy, Vaasa. 168 p.

Kalela EK (1949) Männiköiden ja kuusikoiden juurisuhteista I. Summary: On the horizontal roots in pine and spruce stand I. Acta Forestalia Fennica 57(2), 79 p.

Kieft TL, Ringelberg DB & White DC (1994) Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Applied and Environmental Microbiology 60: 3292-3299

Killham K (1990) Nitrification in coniferous forest soils. Plant and Soil 128: 31-44

Killham K (1994) Soil ecology. Cambridge University Press, Cambridge. 242 p.

Klemedtsson L, Svensson BH & Rosswall T (1988) A method of selective inhibition to distinguish between nitrification and denitrification as sources of nitrous oxide in soil. Biology and Fertility of Soils 6: 112-119

Kloepper JW (1992) Plant growth-promoting rhizobacteria as biological control agents. In: Soil Microbial Ecology. Edited by: Metting FB Jr. Marcel Dekker, New York. p. 255-305

Kowalchuk GA, Stephen JR, De Boer W, Prosser JI, Embley TM & Woldendorp JW (1997) Analysis of ammonia-oxidizing bacteria of the β subdivision of the class *Proteobacteria* in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments. Applied and Environmental Microbiology 63: 1489-1497

Kroppenstedt RM (1985) Fatty acid and menaquinone analysis of actinomycetes and related organisms. In: Chemical Methods in Bacterial Systematics. Edited by: Goodfellow M & Minnikin DE. Academic Press, London. p. 173-199

Laitakari E (1929) Männyn juuristo. Morfologinen tutkimus. Summary: The root system of pine (*Pinus silvestris*). A morphological investigation. Acta Forestalia Fennica 33(2), 380 p.

Laitakari E (1935) Koivun juuristo. Summary: The root system of birch (*Betula verrucosa* and *odorata*). Acta Forestalia Fennica 41(2), 216 p.

Leikola M (1977) Maanmuokkaus ja pintakasvillisuuden torjunta peltojen metsittämisessä. Summary: Soil tilling and weed control in afforestation of abandoned fields. Communicationes Instituti Forestalis Fenniae 88(3), 101 p.

Leyval C & Berthelin J (1993) Rhizodeposition and net release of soluble organic compounds by pine and beech seedlings inoculated with rhizobacteria and ectomycorrhizal fungi. Biology and Fertility of Soils 15: 259-267

Liljeroth E, Van Veen JA & Miller HJ (1990) Assimilate translocation to the rhizosphere of two wheat lines and subsequent utilization by rhizosphere microorganisms at two soil nitrogen concentrations. Soil Biology and Biochemistry 22: 1015-1021

Linderman RG (1988) Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. Phytopathology 78: 366-371

Lodhi MAK & Killingbeck KT (1980) Allelopathic inhibition of nitrification and nitrifying bacteria in a ponderosa pine (*Pinus ponderosa* Dougl.) community. American Journal of Botany 67: 1423-1429

Luo J, White RE, Ball PR & Tillman RW (1996) Measuring denitrification activity in soils under pasture: optimizing conditions for the short-term denitrification enzyme assay and effects of soil storage on denitrification activity. Soil Biology and Biochemistry 28: 409-417

Marschner H, Häussling M & George E (1991) Ammonium and nitrate uptake rates and rhizosphere pH in non-mycorrhizal roots of Norway spruce (*Picea abies* (L.) Karst.). Trees 5: 14-21

Martikainen PJ (1984) Nitrification in two coniferous forest soils after different fertilization treatments. Soil Biology and Biochemistry 16: 577-582

Martikainen PJ (1985) Numbers of autotrophic nitrifiers and nitrification in fertilized forest soil. Soil Biology and Biochemistry 17: 245-248

Martikainen PJ & Palojärvi A (1990) Evaluation of the fumigation-extraction method for the determination of microbial C and N in a range of forest soils. Soil Biology and Biochemistry 22: 797-802

McFee WW & Stone EL Jr (1968) Ammonium and nitrate as nitrogen sources for *Pinus radiata* and *Picea glauca*. Soil Science Society of America Proceedings 32: 879-884

Meharg AA & Killham K (1995) Loss of exudates from the roots of perennial ryegrass inoculated with a range of microorganisms. Plant and Soil 170: 345-349

Meyer FH (1974) Physiology of mycorrhiza. Annual Reviews of Plant Physiology 25: 567-586

Mikola M (1954) Kokeellisia tutkimuksia metsäkarikkeiden hajaantumis-nopeudesta. Summary: Experiments on the rate of decomposition of forest litter. Communicationes Instituti Forestalis Fenniae 43(1), 50 p.

Mikola P (1973) Application of mycorrhizal symbioses in forestry practises. In: Ectomycorrhizae - their ecology and physiology. Edited by: Marks GC & Kozlowski TT. Academic Press, New York. p. 383-411

Mikola P (1985) The effect of tree species on the biological properties of forest soil. National Swedish Environmental Protection Board, Rapport 3017, 27 p.

Miles J & Young WF (1980) The effects on heathland and moorland soils in Scotland and northern England following colonization by birch (*Betula* spp.). Bulletin d'Ecologie (France) 11: 233-242

Millar WN & Casida LE (1970) Evidence for muramic acid in soil. Canadian Journal of Microbiology 16: 299-304

Miller HG (1984) Nutrient cycles in birchwoods. Proceedings of the Royal Society of Edinburgh 85B: 83-96

Miller RM & Jastrow JD (1990) Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. Soil Biology and Biochemistry 22: 579-584

Milliken GA & Johnson DE (1984) Analysis of messy data. Van Nostrand Reinhold Company, New York. 473 p.

Moore DRE & Waid JS (1971) The influence of washings of living roots on nitrification. Soil Biology and Biochemistry 3: 69-83

Mustonen S (1995) Tilastolliset monimuuttujamenetelmät. Yliopistopaino, Helsinki. 205 p.

Myrold DD & Tiedje JM (1986) Simultaneous estimation of several nitrogen cycle rates using ^{15}N: theory and application. Soil Biology and Biochemistry 18: 559-568

Näsholm T, Ekblad A, Nordin A, Giesler R, Högberg M and Högberg P (1998) Boreal forest plants take up organic nitrogen. Nature 393: 914-916

Nichols PD, Stulp BK, Jones JG & White DC (1986) Comparison of fatty acid content and DNA homology of the filamentous gliding bacteria *Vitreoscilla*, *Flexibacter*, and *Filibacter*. Archives of Microbiology 146: 1-6

Nihlgård B (1971) Pedological influence of spruce planted on former beech forest soils in Scania, South Sweden. OIKOS 22: 302-314

Nohrstedt H-Ö (1985) Nonsymbiotic nitrogen fixation in the topsoil of some forest stands in central Sweden. Canadian Journal of Forest Research 15: 715-722

Nohrstedt H-Ö (1988) Nitrogen fixation (C_2H_2-reduction) in birch litter. Scandinavian Journal of Forest Research 3: 17-23

Norton JM & Firestone MK (1991) Metabolic status of bacteria and fungi in the rhizosphere of ponderosa pine seedlings. Applied and Environmental Microbiology 57: 1161-1167

Norton JM & Firestone MK (1996) N dynamics in the rhizosphere of *Pinus ponderosa* seedlings. Soil Biology and Biochemistry 28: 351-362

Nye PH (1981) Changes in pH accross the rhizosphere induced by roots. Plant and Soil 61: 7-26

Nykvist N (1963) Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter. Studia Forestalia Suecica 3, 31 p.

Nylund J-E & Wallander H (1992) Ergosterol analysis as a means of quantifying mycorrhizal biomass. Methods in Microbiology 24: 77-88

O'Leary WM & Wilkinson SG (1988) Gram-positive bacteria. In: Microbial Lipids, Vol 1. Edited by: Ratledge C & Wilkinson SG. Academic Press, London. p. 117-202

Olsson P-A & Wallander H (1998) Interactions between ectomycorrhizal fungi and the bacterial community in soils amended with various primary minerals. FEMS Microbiology Ecology 27: 195-205

Olsson P-A, Bååth E, Jakobsen I & Söderström B (1995) The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycological Research 99: 623-629

Olsson P-A, Bååth E, Jakobsen I & Söderström B (1996) Soil bacteria respond to presence of roots but not to mycelium of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry 28: 463-470

Paavolainen L & Smolander A (1998) Nitrification and denitrification in soil from a clear-cut Norway spruce (*Picea abies*) stand. Soil Biology and Biochemistry 30: 775-781

Paavolainen L, Kitunen V & Smolander A (1998) Inhibition of nitrification in forest soil by monoterpenes. Plant and Soil 205: 147-154

Paré D & Bergeron Y (1996) Effect of colonizing tree species on soil nutrient availability in a clay soil of the boreal mixedwood. Canadian Journal of Forest Research 26: 1022-1031

Parkinson D & Coleman DC (1991) Microbial communities, activity and biomass. Agriculture, Ecosystems and Environment 34: 3-33

Parmelee RW, Ehrenfeld JG & Tate III RL (1993) Effects of pine roots on microorganisms, fauna, and nitrogen availability in two soil horizons of a coniferous forest spodosol. Biology and Fertility of Soils 15: 113-119

Pastor J, Gardner RH, Dale VH & Post WM (1987) Successional changes in nitrogen availability as a potential factor contributing to spruce declines in boreal North America. Canadian Journal of Forest Research 17, 1394-1400

Pennanen T, Fritze H, Vanhala P, Kiikkilä O, Neuvonen S & Bååth E (1998) Structure of a microbial community in soil after prolonged addition of low levels of simulated acid rain. Applied and Environmental Microbiology 64: 2173-2180

Pennanen T, Liski J, Bååth E, Kitunen V, Uotila J, Westman CJ & Fritze H. Structure of the microbial communities in coniferous forest soils in relation to site fertility and stand development stage. Submitted manuscript.

Pietikäinen J & 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

Priha O & Smolander A (1995) Nitrification, denitrification and microbial biomass N in soil from two N-fertilized and limed Norway spruce forests. Soil Biology and Biochemistry 27: 305-310

Purchase BS (1974) Evaluation of the claim that grass root exudates inhibit nitrification. Plant and Soil 41: 527-539

Raison RJ, Connell MJ & Khanna PK (1987) Methodology for studying fluxes of soil mineral N *in situ*. Soil Biology and Biochemistry 19: 521-530

Ranger J & Nys C (1994) The effect of spruce (*Picea abies* Karst.) on soil development: an analytical and experimental approach. European Journal of Soil Science 45: 193-204

Ranta E, Rita H, & Kouki J (1989) Biometria, 2nd edn. Yliopistopaino, Helsinki. 569 p.

Reid CPP, Kidd FA & Ekwebelam SA (1983) Nitrogen nutrition, photosynthesis and carbon allocation into ectomycorrhizal pine. Plant and Soil 71: 415-432

Rice EP & Pancholy SK (1972) Inhibition of nitrification by climax ecosystems. American Journal of Botany 59: 1033-1040

Rikala R & Huurinainen S (1990) Lannoituksen vaikutus kaksivuotisten männyn paakkutaimien kasvuun taimitarhalla ja istutuksen jälkeen. Summary: Effect of fertilization on the nursery growth and outplanting success of two-year-old containerized Scots pine seedlings. Folia Forestalia 745, 16 p.

Rosado AS, Duarte GF, Seldin L & Van Elsas JD (1997) Molecular microbial ecology: a minireview. Revista de Microbiologia 28: 135-147

Rosendahl S and Sen R (1992) Isozyme analysis of mycorrhizal fungi and their mycorrhiza. In: Techniques for the study of ectomycorrhiza. Methods in Microbiology, Vol. 24. Edited by: Norris JR, Read DJ & Varma AK. Academic Press, London. p. 167-194

Rousseau JVD & Reid CPP (1990) Effects of phosphorus and ectomycorrhizas on the carbon balance of loblolly pine seedlings. Forest Science 36: 101-112

Saarsalmi A, Palmgren K & Levula T (1992) Harmaalepän ja rauduskoivun biomassan tuotos ja ravinteiden käyttö energiapuuviljelmällä. Summary: Biomass production and nutrient consumption of *Alnus incana* and *Betula pendula* in energy forestry. Folia Forestalia 797, 29 p.

Saetre P (1998) Decomposition, microbial community structure, and earthworm effects along a birch-spruce soil gradient. Ecology 79: 834-846

Saetre P, Sturensson Saetre L, Brandtberg P-O, Lundkvist H & Bengtsson J (1997) Ground vegetation composition and heterogeneity in pure Norway spruce and mixed Norway spruce - birch stands. Canadian Journal of Forest Research 27: 2034-2042

Scaglia J, Lensi R & Chalamet A (1985) Relationship between photosynthesis and denitrification in planted soil. Plant and Soil 84: 37-43
Schenck NC (1981) Can mycorrhizae control root disease? Plant Disease 65: 230-234
Schimel JP, Jackson LE & Firestone MK (1989) Spatial and temporal effects on plant-microbial competition for inorganic nitrogen in a California annual grassland. Soil Biology and Biochemistry 21: 1059-1066
Scott NA (1998) Soil aggregation and organic matter mineralization in forests and grasslands: plant species effects. Soil Science Society of America Journal 62: 1081-1089
Smith WH (1976) Character and significance of forest tree root exudates. Ecology 57: 324-331
Smith SE & Read DJ (1997) Mycorrhizal symbiosis. 2nd Edition. Academic Press, Cambridge, p. 233-289
Smolander A (1990) *Frankia* populations in soils under different tree species - with special emphasis on soils under *Betula pendula*. Plant and Soil 121: 1-10
Smolander A & Mälkönen E (1994) Microbial biomass C and N in limed soil of Norway spruce stands. Soil Biology and Biochemistry 26: 503-509
Smolander A, Kitunen V, Priha O & Mälkönen E (1995) Nitrogen transformations in limed and nitrogen fertilized soil in Norway spruce stands. Plant and Soil 172: 107-115
Smolander A, Priha O, Paavolainen L, Steer J & Mälkönen E (1998) Nitrogen and carbon transformations before and after clear-cutting in repeteadly N-fertilized and limed forest soil. Soil Biology and Biochemistry 30: 477-490
Söderberg KH & Bååth E (1998) Bacterial activity along a young barley root measured by the thymidine and leucine incorporation techniques. Soil Biology and Biochemistry 30: 1259-1268
Sparling GP & Williams BL (1986) Microbial biomass in organic soils: estimation of biomass C, and effect of glucose or cellulose amendments on the amounts of N and P released by fumigation. Soil Biology and Biochemistry 18: 507-513
Sparling GP, Feltham CW, Reynolds J, West AW & Singleton P (1990) Estimation of soil microbial C by a fumigation-extraction method: use on soils of high organic matter content, and a reassessment of the k_{ec}-factor. Soil Biology and Biochemistry 22: 301-307
Stark JM & Hart SC (1997) High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385: 61-64
Stephen JR, McCaig AE, Smith Z, Prosser JI & Embley TM (1996) Molecular diversity of soil and marine 16S rRNA gene sequences related to β-subgroup ammonia-oxidizing bacteria. Applied and Environmental Microbiology 62: 4147-4154
Strzelczyk E & Bokojska-Burdziej A (1984) Production of auxins and gibberellin-like substances by mycorrhizal fungi, bacteria and actinomycetes from soil and the mycorrhizosphere of pine (*Pinus sylvestris* L.). Plant and Soil 81: 185-194
Strzelczyk E, Kampert M & Michalski L (1985) Production of cytokinin-like substances by mycorrhizal fungi of pine (*Pinus sylvestris* L.) in cultures with and without metabolites of actinomycetes. Acta Microbiologica Polonica 34: 177-186
Strzelczyk E, Dahm H, Kampert M, Pokojska A & Rõzycki H (1987) Activity of bacteria and actinomycetes associated with mycorrhiza of pine (*Pinus sylvestris* L.) Angewandte Botanik 61: 53-64

Timonen S, Jørgensen KS, Haahtela K & Sen R (1998) Bacterial community structure at defined locations of *Pinus sylvestris* - *Suillus bovinus* and - *Paxillus involutus* mycorrhizospheres in dry pine forest humus and nursery peat. Canadian Journal of Microbiology 44: 499-513

Tisdall JM & Oades JM (1982) Organic matter and water-stable aggregates in soils. Journal of Soil Science 33: 141-163

Trolldenier G (1989) Plant nutritional and soil factors in relation to microbial activity in the rhizosphere, with particular emphasis on denitrification. Zeitschrift für Plfanzenernaehrung und Bodenkunde 152: 223-230

Tunlid A & White DC (1992) Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. In: Soil Biochemistry, Vol 7. Edited by: Stotzky G & Bollag J-M. Marcel Dekker Inc., New York. p. 229-262

Turner DP, Sollins P, Lauking M & Rudd N (1993) Availability and uptake of inorganic nitrogen in a mixed old-growth coniferous forest. Plant and Soil 148: 163-174

Van Veen JA, Merckx R & Van de Gejn SC (1989) Plant- and soil related controls of the flow of carbon from roots through the soil microbial biomass. Plant and Soil 115: 179-188

Vance ED, Brookes PC & Jenkinson DS (1987a) Microbial biomass measurements in forest soils: determination of k_c values and tests of hypotheses to explain the failure of the chloroform fumigation-incubation method in acid soils. Soil Biology and Biochemistry 19: 689-696

Vance ED, Brookes PC & Jenkinson DS (1987b) Microbial biomass measurements in forest soils: the use of chloroform fumigation-incubation method in strongly acid soils. Soil Biology and Biochemistry 19: 697-702

Vance ED, Brookes PC & Jenkinson DS (1987c) An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19: 703-707

Verhagen FJM & Laanbroek HJ (1991) Competition for ammonium between nitrifying and heterotrophic bacteria in dual energy-limited chemostats. Applied and Environmental Microbiology 57: 3255-3263

Verhagen FJM & Laanbroek HJ (1992) Effects of grazing by flagellates on competition for ammonium between nitrifying and heterotrophic bacteria in chemostats. Applied and Environmental Microbiology 58: 1962-1969

Verhagen FJM, Duyts H & Laanbroek HJ (1992) Competition for ammonium between nitrifying and heterotrophic bacteria in continuously percolated soil columns. Applied and Environmental Microbiology 59: 3303-3311

Viro PJ (1955) Investigations on forest litter. Communicationes Instituti Forestalis Fenniae 45(6), 65 p.

von Rheinbaben W & Trolldenier G (1983) Influence of mineral nutrition on denitrification on plants in nutrient solution culture. Zeitschrift für Plfanzenernaehrung und Bodenkunde 146: 180-187

von Rheinbaben W & Trolldenier G (1984) Influence of mineral nutrition on denitrification in relation to soil moisture and potassium nutrition. Zeitschrift für Plfanzenernaehrung und Bodenkunde 147: 730-738

Wang X & Zabowski D (1998) Nutrient composition of Douglas-fir rhizosphere and bulk soil solutions. Plant and Soil 200: 13-20

Wardle DA (1992) A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological Reviews 67: 321-358

Weier KL & Macrae IC (1993) Net mineralization, net nitrification and potentially available nitrogen in the subsoil beneath a cultivated crop and a permanent pasture. Journal of Soil Science 44: 451-458

West AW (1986) Improvement of the selective respiratory inhibition technique to measure eukaryote:prokaryote ratios in soils. Journal of Microbiological Methods 5: 125-138

West AW, and Sparling GP (1986) Modifications to the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents. Journal of Microbiological Methods 5: 177-189

Wheatley R, Ritz K & Griffiths B (1990) Microbial biomass and mineral N transformations in soil planted with barley, ryegrass, pea or turnip. Plant and Soil 127: 157-167

White CS (1986) Volatile and water-soluble inhibitors of nitrogen mineralization and nitrification in a ponderosa pine ecosystem. Biology and Fertility of Soils 2: 97-104

White CS (1991) The role of monoterpenes in soil nitrogen cycling processes in ponderosa pine. Biogeochemistry 12: 43-68

Wilkinson SG (1988) Gram-negative bacteria. In: Microbial lipids, Vol 1. Edited by: Ratledge C & Wilkinson SG. Academic Press, London. p. 299-488

Williams BL & Sparling GP (1984) Extractable N and P in relation to microbial biomass in UK acid organic soils. Plant and Soil 76: 139-148

Willison TW & Anderson JM (1991) Denitrification potentials, controls and spatial patterns in a Norway spruce plantation. Forest Ecology and Management 44: 69-76

Wollersheim R, Trolldenier G & Beringer H (1987) Effect of bulk density and soil water tension on denitrification in the rhizosphere of spring wheat (*Triticum vulgare*). Biology and Fertility of Soils 5: 181-187

Yoshinari T & Knowles R (1976) Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochemical and Biophysical Research Communications 69: 705-710

Total number of references: 212

Paper I

I

Priha O. & Smolander A. (1997) Microbial biomass and activity in soil and litter under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at originally similar field afforestation sites. Biology and Fertility of Soils 24: 45-51.

Statement of the contribution of the individual authors in paper entitled

Microbial biomass and activity in soil and litter under *Pinus sylvestris, Picea abies* and *Betula pendula* at originally similar field afforestation sites

by

Outi Priha and Aino Smolander

Finnish Forest Reasearch Institute, P.O.Box 18, FIN-01301 Vantaa, Finland

Which was published 1997 in Biology and Fertility of Soils 24: 45-51

Contributions of the authors listed are:

Outi Priha: Corresponding author. Performing experimental work. Responsible for writing and interpretation of the results.

Aino Smolander: Supervisor of the Ph.D. studies of O. Priha.

Both authors have given their comments of the paper before submission.

Outi Priha

Aino Smolander

Biol Fertil Soils (1997) 24:45–51

ORIGINAL PAPER

O. Priha · A. Smolander

Microbial biomass and activity in soil and litter under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at originally similar field afforestation sites

Received: 2 August 1995

Abstract Microbial biomass C and N, and activities related to C and N cycles, were compared in needle and leaf litter, and in the uppermost 10 cm of soil under the litter layer in Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.) and silver birch (*Betula pendula* L.) stands, planted on originally similar field afforestation sites 23–24 years ago. The ground vegetation was differentiated under different tree species, consisting of grasses and herbs under birch and pine, and mosses or no vegetation with a thick layer of needles under spruce. The C:N ratio of the soils was 13–21 and the soil pH_{CaCl_2} 3.8–5.2. Both showed little variation under different tree species. Microbial biomass C and N, C mineralization, net ammonification, net nitrification and N_2-fixing activity (acetylene reduction) did not differ significantly in soil under different tree species either. Birch leaf litter had a higher pH_{CaCl_2} (5.9) than spruce and pine needle litter (pH 5.0 and 4.8, respectively). The C:N ratio of spruce needles was 30, and was considerably higher in pine needles (69) and birch leaves (54). Birch leaves tended to have the highest microbial biomass C and C mineralization. Spruce needles appeared to have the highest microbial biomass N and net formation of mineral N, whereas formation of mineral N in pine needles and birch leaves was negligible. Microbial biomass C and N were of the same order of magnitude in the soil and litter samples but C mineralization was tenfold higher in the litter samples.

Key words Forest soil · Tree species · *Pinus sylvestris* L. *Picea abies* L. · *Betula pendula* L. · Field afforestation Microbial biomass C · Microbial biomass N · Microbial respiration · Ammonification · Nitrification

O. Priha (✉) · A. Smolander
Finnish Forest Research Institute, PO Box 18, FIN-01301, Finland

Introduction

Trees affect the soil by their litter, root activity and associated microclimate. The structure and decomposability of leaf and needle litter vary, thus affecting the rate of nutrient cycling and the nutrient availability in soil. The waxes of the surface layer and high concentration of phenolic compounds make needle litter difficult to decompose, whereas leaf litter contains more easily leached and decomposed water-soluble compounds (Nykvist 1963). The quantity and quality of root litter, root exudates (Smith 1976) and the takeup of nutrients and water also vary with plant species. Although several studies have compared the effects of different agricultural plants on soil microbiology, studies with different tree species, especially boreal species, are scarce. In a study in Belgium the dominant tree species was more important in determining the biological and chemical fertility of a stand than were the soil texture and climate (Muys and Lust 1992).

The major tree species in Finland are Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.) and silver birch (*Betula pendula* L.). It has been shown that conifers, spruce in particular, may gradually change the soil properties in a disadvantageous direction, whereas birch can maintain or improve the soil properties by raising the pH and enhancing the cycling of nutrients (Gardiner 1968; Miles and Young 1980; Mikola 1985). The mechanism for this possible soil-improving effect of birch is not clear. The aim of this study was to compare C mineralization, ammonification, nitrification, acetylene-reduction and microbial biomass C and N in needle and leaf litter and in soil under pine, spruce and birch planted at originally similar agricultural sites.

Materials and methods

Study sites

Two afforestation experiments established by Leikola (1977) were studied, one at Karttula (62°52′ N, 27°10′ E, 98 m asl) and one at

Maalahti (62°52′ N, 21°33′ E, 15 m asl). Both experiments contained three blocks divided into plots of pine, spruce and birch (randomized block design). The average size of a plot was 0.2 ha. The Karttula experiment was planted in 1970. Block 1 was originally classified as organic (peat) soil and blocks 2 and 4 mineral soil, the soil textural class being silty fine sand (Leikola 1977). The Maalahti experiment was planted in 1971. As in Karttula, block 1 was originally classified as organic soil and blocks 2 and 3 mineral soil, the soil textural class being clay silt. Before planting both sites had been used for growing hay for several years (Leikola 1977). During the 1950–1980 period the effective temperatures sums, with a threshold temperature of +5°C, were 1153 and 1171 degree days, and the mean annual rainfalls 561 and 515 mm for the Karttula and Maalahti experiments, respectively.

Sampling and storage

Samples were taken from Karttula in May 1994 and from Maalahti in July 1994. Twenty soil cores were taken systematically from each plot (0–10 cm layer, core diameter 5.8 cm, 0.5–1.0 m from the nearest tree). These were combined to give one composite sample per plot. After 1 week storage at +6°C, green plant material was removed and the samples were sieved (mesh size 5.7 mm) and stored at +6°C in plastic bags. From the Karttula experiment brown needle and leaf litter was collected from the ground of all plots in September 1994. Litter samples were air dried at room temperature for 2 days and stored in paper bags at +6°C.

The soil samples were analysed 1–4 weeks after sieving and litter samples within 2 weeks of sampling. Litter samples were not ground for the analyses, except for total C and N determination. For biological measurements, 3–6 g dm (dry matter) soil was used, adjusted to 60% of the water-holding capacity (WHC), or 1 g dm litter with 3–5 ml water added. Biological determinations were carried out using three replicates, physical and chemical determinations with two replicates.

Physical and chemical characteristics

The dry matter content of soil was determined by drying samples for 18–24 h at 105°C. Soil organic matter content was measured as loss on ignition from the dried samples at 550°C for 4 h. WHC was measured by soaking the soil samples in water for 2 h and then draining for 2 h. Total organic C and total N were determined using an automated CHN analyser (Leco CHN-600). Soil pH was measured in 3:5 v:v soil:water or soil:0.01 *M* $CaCl_2$ suspensions. Dry matter content of the litter samples was determined by drying the samples for 18–24 h at 60°C. Total organic C and total N were determined as above. Litter pH was measured using 1-g-dm litter samples with 30 ml water or 0.01 *M* $CaCl_2$.

Fumigation-extraction

The fumigation-extraction method was essentially that of Vance et al. (1987). Briefly, soil samples were fumigated for 24 h at 28°C with ethanol-free chloroform vapour. The fumigated and the unfumigated control samples were extracted with 0.5 *M* K_2SO_4 (30 min, 200 rpm). The extract was filtered through a 0.45 μm membrane filter (Ion Acrosdisc, Gelman Sciences) for C analysis and through Schleicher & Schuell 589^3-filter papers for N analysis. Total organic C was determined from the extracts with a total organic carbon analyser (Shimadzu TOC-5000) using potassium biphthalate as a standard. Total N was determined colorimetrically with a flow injection analyser (Tecator FIAstar 5012 Analyzer+5042 Detector).

Substrate-induced and basal respiration

Substrate-induced respiration was essentially determined according to West and Sparling (1986). Soil and litter samples were conditioned at room temperature overnight in 125-ml glass bottles closed with rubber septa. A glucose-water solution was added to soil samples to give a soil moisture content of 60% of the WHC, and a glucose concentration of 15 mg ml^{-1} soil water. Five millilitres glucose-water solution was added to litter samples to give a final glucose concentration of 30 mg ml^{-1} soil water. These concentrations were found to be optimal in preliminary experiments (results not shown). After glucose addition, samples were allowed to stabilize for 0.5 h. The bottles were then aerated, closed with rubber septa and incubated at 22°C in a water bath for 2 h. The CO_2 evolved was measured by gas chromatography (Varian 3600) equipped with a thermal conductivity detector and a Megapore GS-Q column (J & W Scientific) 30 m in length, using He (6.4 ml min^{-1}) as carrier gas. The temperatures of the detector, injector and oven were 150°, 120° and 30°C, respectively.

To measure basal respiration, soil samples were incubated at 14°C in 125-ml glass bottles for 2 weeks and litter samples for 1 week. The CO_2 evolved, over 28–48 h (soil) and 18–24 (litter) periods, was measured as above. Samples were aerated between measurements. The results given are means of three measurements.

Incubation experiments to study N transformations

Soil and litter samples were incubated at 14°C for 40 days in 125-ml glass bottles. The bottles were covered with foil and the moisture content adjusted weekly. Samples were then extracted with 40 ml 1 *M* KCl (2 h, 200 rpm) and filtered through Schleicher & Schuell 589^3-filter papers; NH_4^+-N and (NO_2^-+NO_3^-)-N were subsequently determined from the extracts with a flow injection analyser (details above). To calculate net ammonification and nitrification, initial NH_4^+-N and (NO_2^-+NO_3^-)-N concentrations, from non-incubated samples, were subtracted from final (postincubation) NH_4^+-N and (NO_2^-+NO_3^-)-N concentrations.

N_2-fixing activity (acetylene reduction assay)

Soil and litter samples were incubated at 14°C in 125-ml glass bottles under 10 kPa partial pressure of acetylene. Endogenous ethylene release was measured without acetylene addition. The potential nitrogenase activity was determined by adding a glucose-water solution as in substrate-induced respiration. The ethylene evolved was measured after 1 and 2 days incubation with a gas chromatograph (Varian 3600), equipped with a flame ionization detector and a 3-mm-outside-diameter×2-m stainless steel column packed with Porapak N, using He (40 ml min^{-1}) as carrier gas. The temperatures of the detector, injector and oven were 180°, 120° and 70°C, respectively.

Statistical analyses

Differences in the measured characteristics of the soil samples of different tree species were compared with two-way analysis of variance using block∗tree species as the error term (Ranta et al. 1989). Litter samples were compared with one-way analysis of variance. The results were log-transformed if necessary to fulfil the assumptions of variance analysis. Correlation of fumigation-extraction derived and substrate-induced respiration derived microbial biomass C was determined by Pearson's correlation coefficient.

Results

Results are given per unit total organic C (C_{org}). The coefficient of variation (CV%) of the three replicates was, in most cases, <10 for soil samples and <20 for litter samples. In the ammonification and nitrification incubation studies, the CV% of the litter samples was larger (30–40%). A significance level of 95% (*P*=0.05) was used.

Table 1 Some soil characteristics of the experimental sites (*dm* dry matter)

Experiment and block No.	Plot	Organic matter (% of dm)	Organic C (% of dm)	Total N (% of dm)	C:N ratio	pH (H_2O)	pH ($CaCl_2$)	Initial NH_4^+-N ($\mu g\ g^{-1}\ C_{org}$)	Initial (NO_2^-+NO_3^-)-N ($\mu g\ g^{-1}\ C_{org}$)
Karttula									
1	Pine	25	12.6	0.64	20	5.9	5.1	97	204
	Spruce	27	13.7	0.71	19	5.4	4.7	145	110
	Birch	34	19.8	0.93	21	5.8	5.2	100	110
2	Pine	12	5.7	0.33	17	5.3	4.5	170	185
	Spruce	14	7.2	0.39	18	5.4	4.5	216	56
	Birch	12	6.1	0.35	17	5.5	4.6	228	106
4	Pine	12	5.4	0.30	18	5.8	5.0	158	359
	Spruce	12	5.6	0.32	17	5.5	4.8	225	164
	Birch	11	4.9	0.29	17	5.8	5.0	207	260
Maalahti									
1	Pine	36	18.6	1.12	17	4.6	3.9	68	255
	Spruce	48	26.0	1.42	18	4.5	3.8	96	196
	Birch	37	19.1	1.10	17	4.7	4.0	151	150
2	Pine	21	10.7	0.70	15	4.9	4.1	102	235
	Spruce	25	11.6	0.86	13	4.7	4.1	149	198
	Birch	28	13.1	0.89	15	4.5	3.8	85	194
3	Pine	22	13.5	0.81	17	4.5	4.0	119	105
	Spruce	17	8.9	0.60	15	4.5	3.8	190	93
	Birch	21	10.9	0.68	16	4.7	3.9	160	140

Table 2 Some characteristics of the Karttula litter samples. Values are means of plots, standard deviations in parentheses (*dm* dry matter)

Plot	Organic C (% of dm)	Total N (% of dm)	C:N ratio	pH (H_2O)	pH ($CaCl_2$)	Initial NH_4^+-N ($\mu g\ g^{-1}\ C_{org}$)	Initial (NO_2^-+NO_3^-)-N ($\mu g\ g^{-1}\ C_{org}$)
Pine	52.7 (0.2)	0.78 (0.15)	69 (15)	5.2 (0.2)	4.8 (0.2)	38 (15)	0 (0)
Spruce	44.8 (2.6)	1.52 (0.16)	30 (2)	5.4 (0.4)	5.0 (0.4)	453 (141)	46 (40)
Birch	51.9 (0.7)	0.97 (0.12)	54 (6)	6.2 (0.1)	5.9 (0.1)	24 (24)	0 (0)

Physical and chemical characteristics

Tree species did not affect organic matter content, C:N ratio and pH of the soils (Table 1). The C:N ratio of litter varied considerably, being lowest in spruce needles and highest in pine needles (Table 2). The pH of the birch leaves was on average 1 unit higher than in spruce and pine needles.

Fumigation-extraction C, substrate-induced and basal respiration

Tree species did not affect the flush of C from fumigation (Fig. 1a). Substrate-induced respiration did not differ significantly under different tree species either, although in Karttula it was highest under birch in each block (Fig. 1b). If converted to microbial biomass C, the values from fumigation-extraction ranged from 8.3 to 19.2 mg g^{-1} C_{org} (using the equation of Martikainen and Palojärvi 1990) and those from substrate-induced respiration from 6.9 to 21.3 mg g^{-1} C_{org} (using the equation of Anderson and Domsch 1978). Microbial biomass C calculated from both methods correlated significantly (r=0.95). Basal respiration at the spruce plots was significantly higher than at the pine plots in the soil from the Karttula experiment (Fig. 1c). It was also higher in birch plots, but not significantly. In Maalahti there was no tree species effect.

In the Karttula litter samples birch leaves gave higher flushes of C than pine and spruce needles, but because of the high variation the differences were not statistically significant (Fig. 2a). Substrate-induced respiration was significantly lowest in pine needles (Fig. 2b). Birch leaves had the highest substrate-induced respiration, but the difference from spruce needles was not significant. If the soil conversion factors were used, the fumigation-extraction derived microbial biomass C of the litter samples was 4.5–21.6 mg g^{-1} C_{org} and the substrate-induced respiration derived 19–66 mg g^{-1} C_{org}, and the correlation was lower than in the soil samples (r=0.73). Basal respiration was also highest in birch leaves, but not significantly so (Fig. 2c).

Fumigation-extraction N

The flush of extractable N from fumigation was not affected by tree species (Fig. 3). If converted to microbial

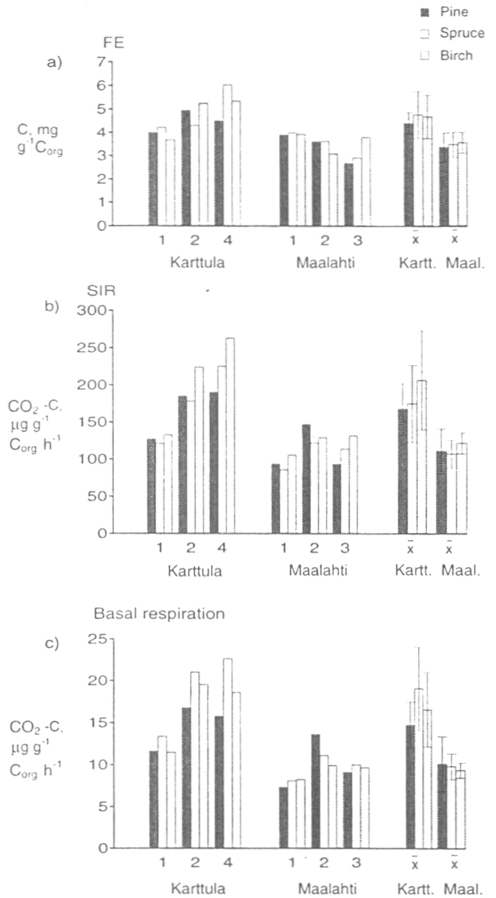


Fig. 1 Soil samples. **a** The flush of extractable C from fumigation. **b** CO_2-C evolved from substrate-induced respiration. **c** CO_2-C evolved from basal respiration. $\bar{x}$ mean of plots, *bars* show standard deviations

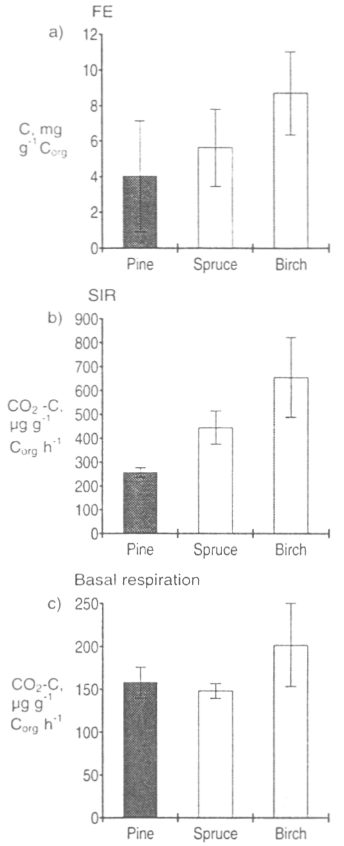


Fig. 2 Karttula litter samples. **a** The flush of extractable C from fumigation. **b** CO_2-C evolved from substrate-induced respiration. **c** CO_2-C evolved from basal respiration. *Values* are means of plots, *bars* show standard deviations

biomass N, the values were 1.3–3.5 mg g^{-1} C_{org} (using the equation of Martikainen and Palojärvi 1990). In the litter samples spruce needles gave the highest and pine needles the lowest N flushes, but the difference were not statistically significant (Fig. 4). Using the soil conversion factor, the microbial biomass N of litter samples was 0.4–2.8 mg g^{-1} C_{org}.

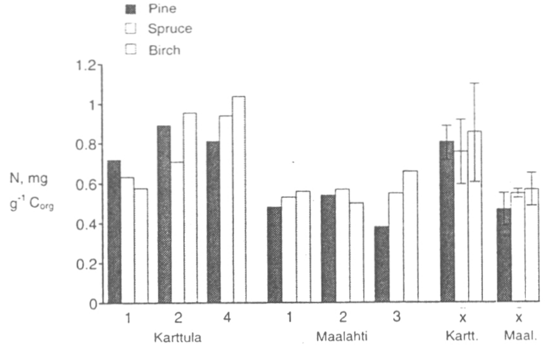


Fig. 3 The flush of extractable N from fumigation of the soils samples. $\bar{x}$ mean of plots, *bars* show standard deviations

Ammonification, nitrification and nitrogenase activity

In Karttula, the soil under pine contained significantly less NH_4^+-N and significantly more $(NO_2^-+NO_3^-)$-N than the soils under birch and spruce (Table 1). The same was true for Maalahti, but the differences were not significant.

During incubation, $(NO_2^-+NO_3^-)$-N was produced in considerable amounts in all soils, whereas NH_4^+-N accumu-

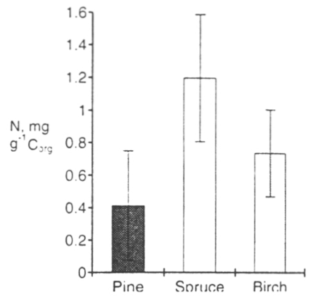


Fig. 4 The flush of extractable N from fumigation of the Karttula litter samples. *Values* are means of plots, *bars* show standard deviations

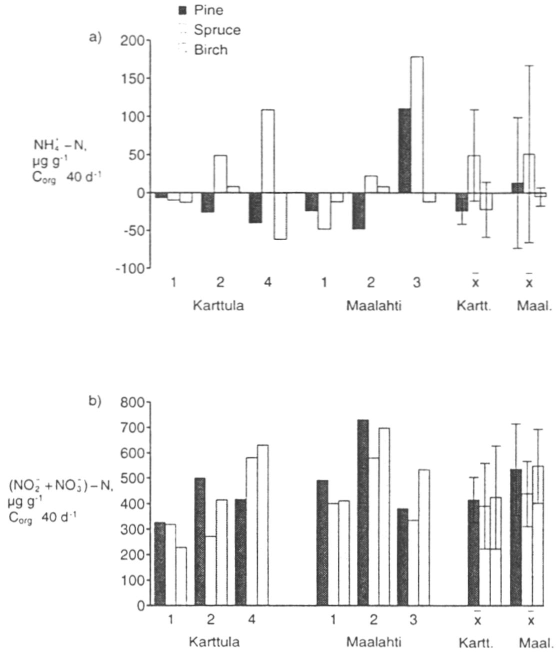


Fig. 5a, b Net ammonification and net nitrification during a 40-day incubation of the soil samples. $\bar{x}$ mean of plots, *bars* show standard deviations

lated in lower amounts, or even decreased (Fig. 5a, b). There were no significant differences in the accumulation of NH_4^+-N or $(NO_2^-+NO_3^-)$-N in soil under different tree species, although the soil under spruce tended to contain more NH_4^+-N and less $(NO_2^-+NO_3^-)$-N than the other soils, both initially and after incubation.

Spruce needles contained the highest initial amounts of NH_4^+-N, whereas the amounts in pine needles and birch leaves were negligible (Table 2). There were also considerable amounts of $(NO_2^-+NO_3^-)$-N in spruce needles, but no detectable levels in pine needles or birch leaves. The same was true for NH_4^+-N and $(NO_2^-+NO_3^-)$-N production during

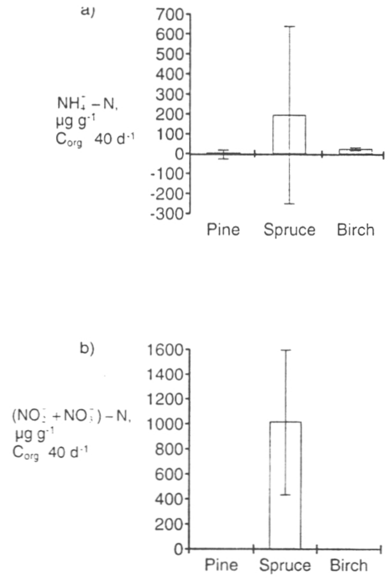


Fig. 6a, b Net ammonification and net nitrification during a 40-day incubation of the litter samples. *Values* are means of plots, *bars* show standard deviations

incubation; spruce needles has the highest production of both, and there was no detectable $(NO_2^-+NO_3^-)$-N production in pine needles or birch leaves (Fig. 6a, b).

No acetylene reduction was detected in the soil or litter samples even when glucose was added.

Proportions of microbial C and N of soil C and N and the metabolic quotients (qCO$_q$)

Calculated from the converted values, the proportion of microbial C was on average 1.5%, and 1.0% of total organic C, and the proportion of microbial N 4.6%, and 2.5% of total N in Karttula and Maalahti soils, respectively (Table 3). The soil microbial C:N ratio varied between 5.7 and 6.8 at both sites. The metabolic quotient qCO_2 determining the specific respiration rate as CO_2-C produced per unit microbial biomass C (expressed as $\times 10^{-3}$ h^{-1}) ranged from 0.94 to 1.29. No consistent effect of tree species was seen in these ratios.

The proportions of microbial C of total organic C, and microbial N of total N, were lowest in pine needles and highest in birch leaves (Table 3). The microbial C:N ratio in spruce needles was half that of pine needles and birch leaves. The qCO_2 was highest in pine needles, both when determined with fumigation-extraction and substrate-induced respiration.

Table 3 Proportions of microbial C and N of soil C and N and metabolic quotients ($q\,CO_2$). Values are means of plots, standard deviations in parentheses (C_{mic} microbial biomass C, *FE* fumigation-extraction, *SIR* substrate-induced respiration, N_{mic} microbial biomass N, $q\,CO_2$ CO_2-C respired, $C_{mic} \times 10^{-3} \times h^{-1}$)

	Plot	C_{mic} (% of C_{org}) (FE)	C_{mic} (% of C_{org}) (SIR)	N_{mic} (% of N_{tot})	C_{mic}:N_{mic} (FE)	$q\,CO_2$ (FE)	$q\,CO_2$ (SIR)
Karttula	Pine	1.5 (0.3)	1.4 (0.3)	4.7 (0.8)	5.7 (0.0)	1.00 (0.05)	1.09 (0.06)
soil	Spruce	1.5 (0.4)	1.4 (0.4)	4.3 (1.0)	6.2 (0.2)	1.29 (0.17)	1.35 (0.11)
	Birch	1.5 (0.5)	1.7 (0.5)	4.7 (1.4)	5.8 (0.4)	1.13 (0.14)	1.00 (0.11)
Maalahti	Pine	1.0 (0.1)	0.9 (0.2)	2.3 (0.3)	6.8 (0.6)	1.04 (0.26)	1.11 (0.12)
soil	Spruce	1.0 (0.1)	0.9 (0.2)	2.5 (0.3)	6.3 (0.9)	0.96 (0.09)	1.12 (0.04)
	Birch	1.0 (0.1)	1.0 (0.1)	2.6 (0.4)	6.2 (0.5)	0.94 (0.13)	0.94 (0.03)
Karttula	Pine	0.8 (0.6)	2.1 (0.1)	5.9 (3.6)	9.5 (1.5)	25.2 (14.9)	7.7 (1.0)
litter	Spruce	1.2 (0.4)	3.6 (0.5)	7.1 (2.1)	4.9 (0.8)	13.9 (5.4)	4.2 (0.7)
	Birch	1.7 (0.4)	5.3 (1.3)	8.0 (2.1)	11.8 (1.3)	11.7 (1.6)	3.9 (0.7)

Discussion

The ground vegetation was already differentiated under pine, spruce and birch planted at originally similar field afforestation sites 23–24 years ago, containing herbs and grasses under birch and pine and either mosses or no ground vegetation with a thick layer of needles under spruce. The ground vegetation of birch and spruce was typical but shrubs or lichens would usually be found under pine, which naturally grows on poor soils.

Litter samples varied in their microbial biomass and activities. Birch leaves appeared to have the highest microbial biomass C and C mineralization, and pine needles the lowest microbial biomass C but highest metabolic quotient ($q CO_2$) (Fig. 2, Table 3). It has been shown that initially more easily decomposable water-soluble substrates such as sugars, organic acids and amino acids are released from birch leaves than spruce and pine needles (Nykvist 1963; Berg and Wessén 1984). Birch leaves seemed to have a big potentially active microbial population responding quickly to easy substrates (glucose). However, without energy addition the microbial population in pine needles was most active with the highest $q CO_2$ values. Microbial biomass C, when measured by fumigation-extraction, was of the same order of magnitude in the soil and litter samples, whereas substrate-induced respiration was higher in the litter samples, and basal respiration was, on average, 10 times higher in the litter samples (Figs. 1, 2). The $q CO_2$ was also considerably higher in the litter than in the soil samples (Table 3). This indicated the microbial population in litter was generally more active in C mineralization and metabolized glucose more preferentially than the soil microbial population. This seems plausible as Nykvist (1963) found glucose and fructose to be the most abundant sugars in extracts of pine, spruce and birch litter.

It was surprising that the proportion of N in spruce needles was significantly higher and the C:N ratio lower than in birch leaves or pine needles (Table 2). One reason might be that the spruce needles were older than pine needles and birch leaves. The C:N ratio has been seen to decrease with increasing decomposition (Berg and Wessén 1984; Mikola 1955). Mikola (1955) found that the N concentration increases in 2 years of advancing decomposition from 0.45% to 0.66% in pine needle litter, from 0.95% to 1.23% in spruce needle litter and from 0.97% to 1.50% in birch leaf litter. The microbial C:N ratio was also significantly lowest in spruce needles (Table 3). It was not likely that losses of N through denitrification would have caused the negligible net formation of mineral N in pine needles and birch leaves (Fig. 6), because anaerobic conditions did not prevail in the incubation. In the field, N might be intensively taken up by grasses, which were only present in the pine and birch plots.

In spite of the clear differences in the ground vegetation and in the microbiological characteristics of the litter samples, no differential effect of the tree species on soil pH, C:N ratio, microbial biomass C and N or C and N mineralization rates in 23–24 years was seen, in the originally organic soils, or in the mineral ones (Table 1, Figs. 1, 3, 5). Mikola (1985) studied 30- to 50 year-old spruce and birch forests growing on originally approximately similar sites and found no differences in soil respiration. In the birch plots, cellulose decomposition and pH were higher and C:N ratio lower than in the spruce plots, but only in surface soil (0–1.5 cm) (Mikola 1985). As we sampled soil to the depth of 10 cm, the possible differences in the surface soil, where the effect of leaf and needle litter starts, could not be seen. However, the density of fine roots and thus also the amount of root litter and root exudates was large in the mineral soil at this depth.

There is not much microbiological data from field afforestation sites. Microbial biomass and respiration in these sites were of the same order of magnitude as in the lower part of the humus layer of natural coniferous soils in Finland but higher than in the mineral soil below (Smolander and Mälkönen 1994). The proportions of microbial biomass C and N from total organic C and total N (Table 3) were within the range indicated by Martikainen and Palojärvi (1990), who found the corresponding values for coniferous, deciduous and arable soils to be 1.2%, 1.1% and 1.4% of total C and 5.9%, 3.4% and 2.5% of total N, respectively. All of the soils contained NO_3^--N both initially and after incubation (Table 1, Fig. 5b). Finnish coniferous forest soils generally show no nitrification activity unless managed with fertilization (e.g. Martikainen 1984; Smolander et al. 1995) or clear-cutting (Smolander et al. 1994). The high nitrification activity shown here was probably due to the sites being former fields, with a higher pH (at least in Karttula) and lower C:N ratio and thus higher nitrogen availability than in natural Finnish coniferous forest soils. Nitrogen addition and pH increase can induce nitrification in acid Finnish forest soils (e.g. Smolander et al. 1995).

Fumigation-extraction and substrate-induced respiration results, except where stated, were shown without the use of conversion factors. The reason for this is a lack of a suitable conversion factor for these kinds of soils and litters. Several studies have found similar patterns for soil microbial biomass C measured with different methods, but have found the absolute values to vary considerable (e.g. West et al. 1986; Kaiser et al. 1992). In our study, however, the methods gave very similar values for the soil samples.

When using the conversion factors of soils for the litter samples, the microbial biomass C from substrate-induced respiration was on average 3 times higher than from fumigation-extraction. This indicates that either chloroform does not lyse the microbes in litter samples as efficiently as in soil samples, which seems unlikely, or the microbial population in litter samples consists of microbes able to metabolize glucose more preferentially than the ones in soils as discussed earlier.

No acetylene reduction activity was detected in the soil or litter samples. Lack of a suitable energy source cannot be the reason as glucose addition did not elicit any activity. Nohrstedt (1985, 1988) found high acetylene reduction activities in birch leaf litter. Our results merely indicate that at the sampling time, under the conditions used in the measurement, there was no nitrogenase activity.

In conclusion, the microbiological characteristics in needle litters of pine and spruce and leaf litter of birch varied, C mineralization and microbial biomass C being highest in birch leaves and net formation of mineral N and microbial biomass N in spruce needles. However, tree species had no clear differential effect on the soil organic matter content, C:N ratio or pH, or on soil microbial biomass C and N, or C and N mineralization rates after 23–24 years.

Acknowledgements We are grateful for Orlando Rutter and Jonathan Steer for help with the field work and Tuula Aarnio, Dr. Hannu Frize, Prof. Eino Mälkönen and Taina Pennanen for comments on the manuscript. We also thank Jonathan Steer for revising the language. The Academy of Finland supported this work.

References

Anderson JPE, Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol Biochem 10:215–221

Berg B, Wessén B (1984) Changes in organic-chemical components and ingrowth of fungal mycelium in decomposing birch leaf litter as compared to pine needles. Pedobiologia 26:285–298

Gardiner AS (1968) The reputation of birch for soil improvement. For Comm Res Dev Pap 67:1–9

Kaiser E-A, Mueller T, Joergensen RG, Insam H, Heinemeyer O (1992) Evaluation of methods to estimate the soil microbial biomass and the relationship with soil texture and organic matter. Soil Biol Biochem 24:675–683

Leikola M (1977) Maanmuokkaus ja pintakasvillisuuden torjunta peltojen metsittämisessä. English summary: Soil tilling and weed control in afforestation of abandoned fields. Commun Inst For Fenn 88.3:1–101

Martikainen PJ (1984) Nitrification in two coniferous forest soils after different fertilization treatments. Soil Biol Biochem 16:577–582

Martikainen PJ, Palojärvi A (1990) Evaluation of the fumigation-extraction method for the determination of microbial C and N in a range of forest soils. Soil Biol Biochem 22:797–802

Mikola M (1955) Kokeellisia tutkimuksia metsäkarikkeiden hajaantumisnopeudesta. English summary: Experiments on the rate of decomposition of forest litter. Commun Inst For Fenn 43.1:1–50

Mikola M (1985) The effect of tree species on the biological properties of forest soil. Nat Swed Env Protect Board 3017:1–29

Miles J, Young WF (1980) The effects on heathland and moorland soils in Scotland and northern England following colonization by birch (*Betula* spp.). Bull Ecol (France) 11:233–242

Muys B, Lust N (1992) Inventory of the earthworm communities and the state of litter decomposition in the forests of Flanders, Belgium, and its implications for forest management. Soil Biol Biochem 24:1677–1681

Nohrstedt H-Ö (1985) Nonsymbiotic nitrogen fixation in the topsoil of some forest stands in central Sweden. Can J For Res 15:715–722

Nohrstedt H-Ö (1988) Nitrogen fixation (C_2H_2-reduction) in birch litter. Scand J For Res 3:17–23

Nykvist N (1963) Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter. Stud For Suec 3:1–31

Ranta E, Rita H, Kouki J (1989) Biometria, 2nd edn. Yliopistopaino, Helsinki

Smith WH (1976) Character and significance of forest tree root exudates. Ecol 57:324–331

Smolander A, Mälkönen E (1994) Microbial biomass C and N in limed soil of Norway spruce stands. Soil Biol Biochem 26:503–509

Smolander A, Mälkönen E, Helmisaari H-S, Kitunen V, Kukkola M, Martikainen PJ, Priha O (1994) Typen sitoutuminen ja mineralisaatio typellä kuormitetuissa kuusikoissa. Metsäntutkimuslaitoksen tiedonantoja 527:224–232

Smolander A, Kitunen V, Priha O, Mälkönen E (1995) Nitrogen transformations in limed and nitrogen fertilized soil in Norway spruce stands. Plant and Soil 172:107–115

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

West AW, Sparling GP (1986) Modifications to the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents. J Microb Meth 5:177–189

West AW, Sparling GP, Grant WD (1986) Correlation between four methods to estimate total microbial biomass in stored, air-dried and glucose-amended soils. Soil Biol Biochem 18:569–576

Paper II

II

Priha O. & Smolander A. (1999) Nitrogen transformations in soil under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. Soil Biology and Biochemistry 31: 965-977 (in press).

Statement of the contribution of the individual authors in paper entitled

Nitrogen transformations in soils under *Pinus sylvestris, Picea abies* and *Betula pendula* at two forest sites

by

Outti Priha and Aino Smolander

Finnish Forest Reasearch Institute, P.O.Box 18, FIN-01301 Vantaa, Finland

Which has been accepted for publication in Soil Biology and Biochemistry 4 December, 1998.

Contributions of the authors listed are:

Outi Priha: Corresponding author. Performing experimental work. Responsible for writing and interpretation of the results.

Aino Smolander: Supervisor of the Ph.D. studies of O. Priha.

Both authors have given their comments of the paper before submission.

Outi Priha

Aino Smolander

PERGAMON

Soil Biology and Biochemistry 00 (1999) 1–13

Nitrogen transformations in soil under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites

Outi Priha *, Aino Smolander

Finnish Forest Research Institute, P.O. Box 18, FIN-01301 Vantaa, Finland

Accepted 4 December 1998

Abstract

Microbial biomass N, net ammonification, net nitrification, denitrification potential, numbers of nitrifiers and the pH-dependency of nitrification were measured from the humus layer and mineral soil layers in adjacent stands of Scot~~ch~~s pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) Karst.) and silver birch (*Betula pendula* L.). The trees had been established at two forest sites of different fertility approximately 60 years ago. The aim was to see whether the microbial and chemical characteristics of the soils differed under different tree species. The soil pH(H_2O) varied from 3.8 to 5.0 and was lowest in spruce soil at both sites in all soil layers. Microbial biomass N, ammonification, nitrification and denitrification all differed in soils of pine, spruce and birch. The flush of N from fumigation varied from 36 to 67 μg N cm^{-3} fresh soil in the humus layer, and from 13–50 μg cm^{-3} in the mineral soil layers. Denitrification potential with added nitrate was 2–29 ng N_2O-N cm^{-3} soil h^{-1} in the humus layer and 0–28 ng in the mineral soil layers. Both tended to be lowest under spruce and highest under birch, at the fertile site in all soil layers and at the less fertile site in the humus layer. In the mineral soil layers of the fertile site and in the humus layer and upper mineral soil layer of the less fertile site the content of mineral N was highest under birch. Different populations of nitrifiers existed in the soils, regarding numbers, activity and pH-dependency. Only the nitrifier community in pine humus layer from the fertile site was adapted to acidic (pH 4.1) conditions. In an aerobic soil suspension the cumulative nitrate production of it was 32 μg cm^{-3} soil in three weeks, compared to negligible production in the other soils. When the pH of the suspensions was raised to 6.0, all soils from the fertile site produced nitrate, but the production ~~in~~ at the less fertile site was still negligible. Higher C-to-N ratios probably explained the low nitrification activity and numbers of nitrifiers at the less fertile site. Thus, there were differences in N transformations under pine, spruce and birch, but the changes depended also on the fertility of the site.

1. Introduction

The dominant tree species ~~are~~ is important in determining the biological and chemical fertility of a stand (Muys and Lust, 1992). A knowledge of the effects of different tree species can be utilized in the selection of tree species or a mixture of species, in a certain stand. In our previous study we compared microbial and chemical characteristics in soil (0–10 cm under the litter layer) and litter under Scot~~ch~~s pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) Karst.) and silver birch (*Betula pendula* L.) planted at originally similar field afforestation sites 23–24 yr ago (Priha and Smolander, 1997). The ground vegetation had already differentiated under different tree species. The microbial characteristics of the litters varied, C mineralization and microbial biomass C being highest in birch leaves and net mineralization of N and microbial biomass N in spruce needles. Tree species had not, however, had a clear differential effect on the soil characteristics. On one hand, a longer time is possibly needed for trees to cause any changes in soil, and the effect can be limited to certain soil layers only. On the other hand, the possible effect is also bound to depend on the properties of the original site.

* Corresponding author. Tel.: + 358-9-8570/5652, fax: + 358-9-8570-2575, e-mail: outi.priha@metla.fi.

0038-0717/99/$19.00
PII: S0038-0717(99)00006-1

Nitrogen is one of the key elements in forest ecosystems, especially in northern nitrogen-limited forests. The humus N concentration was the soil chemical variable best correlated to site index in over 800 pine and spruce sites in southern Finland (Tamminen, 1993). Microbes are mainly responsible for the transformation of N in soils, and can either mineralize N making it thus available to trees, or immobilize it. Trees assimilate N, but also support microbes with N by their root and leaf litter and root exudates. Thus, the interaction between trees and soil microbes can be either beneficial, competitive, or both, and the situation is likely to vary between different tree species and sites.

In our study microbial biomass N, net ammonification, net nitrification, numbers of nitrifiers, the pH-dependency of nitrification, denitrification potential with or without an added substrate and denitrification enzyme activity were compared in soils under pine, spruce and birch established at two forest sites of different fertility approximately 60 yr ago. The aim was to obtain information of the differences in soils under the three tree species at the two sites of different fertility, and to see whether the changes were different in different layers of soil.

2. Materials and methods

2.1. Study sites

Two field experiments were studied, one at Punkaharju and one at Uurainen (Table 1). Both experiments contained one plot of each tree species (pine, spruce and birch) adjacent to each other, the average size of a plot being 0.5 ha. The Punkaharju experiment was established in 1931. According to a Finnish forest site type classification, which is based on the ground vegetation of the site, Punkaharju is a fertile *Oxalis acetocella–Vaccinium myrtillus* type (OMT) (Cajander, 1949; Beuker, 1994). The Uurainen experiment was ~~planted~~ established in 1936–1940, and is less fertile than Punkaharju, classified as a *Vaccinium vitis–ideae* type (VT) (Cajander, 1949; Jaakko Rokkonen, personal communication). Approximately 11% of forest area in Finland belong to the OMT-type and 33% to the VT-type (Finnish Statistical Yearbook of Forestry, 1997).

2.2. Sampling and storage

Samples were taken in July 1995 for other analyses and in June 1996 for determination of denitrification enzyme activity, numbers of nitrifiers and pH-dependency of nitrification. Twenty soil cores were taken systematically from each plot (to 10 cm depth, core di-

Table 1
General characteristics of the experimental sites. Forest site type classification according to Cajander (1949). Meteorological data are from years 1961–1990 (Climatological Statistics in Finland 1961–1990, 1991)

Site	Forest site type	Geographical location longitude/latitude	Altitude (m)	Previous land use	Year of regeneration	Mean annual temperature (°C)	Mean annual rainfall (~~mM~~ mm)	Soil type	Soil texture	Humus type	Number of thinnings until 1995
Punkaharju	OMT = *Oxalis acetocella–Vaccinium myrtillus*	61°48'N/29°18'E	95	forest site, clear-cut 1926–27	1931	3	580	Podzol	stony till	Mor	6, last 1981
Uurainen	VT = *Vaccinium vitis-ideae*	62°25'N/25°29'E	185	forest site, forest fire, 1933	1936–1940	3	610	Podzol	sandy till	Mor	2, last 1989

ameter 5.8 cm, 0.5–1 m from the nearest tree). A 20-m buffer strip was left between adjacent plots. The cores were transferred to the laboratory and divided into humus layer (FH), 0–3 cm mineral soil layer and 3–6 cm mineral soil layer, which were then combined in a depthwise manner. The soil profile of the VT-site represented a typical podzol, where the soil layers were clearly separated, but at the OMT-site soil layers were more mixed, and mineral soil contained a high proportion of organic matter. From samples taken in 1996, only the humus layer and the 0–3 cm mineral soil layer were separated. Green plant material and coarse roots were removed and the samples were sieved (mesh size 5.7 mm) and stored at +4°C in plastic bags for 1–2 weeks before the analyses. Measurements of nitrification and denitrification potentials, and nutrient analyses were made from frozen (−18°C) soil samples.

2.3. Physical and chemical analyses

The dry matter (d.m.) content of the soil was determined by drying the samples for 18–24 h at 105°C. Soil organic matter content was measured as loss on ignition from the dried samples at 550°C for 4 h. Water-holding capacity (WHC) was measured by soaking the soil samples in water for 2 h and then draining for 2 h. The fresh bulk density of the soil was determined by weighing 50 ml of soil. Soil pH was measured in 3:5 v:v soil: H_2O suspensions.

Total C and N were determined using an automated CHN analyzer (Leco CHN-600). For determination of other total nutrients from the organic matter (P, K, Ca, Mg) the soil samples (2–3 g d.m.) were ignited at 550°C and the ash extracted with 100 ml 0.2 M HCl. For determination of soluble P and exchangeable K, Ca, Mg, Na, Al, Fe and Mn, fresh soil samples (7.5 g humus or 17 g mineral soil) were extracted with 150 ml 0.5 M CH_3COONH_4 (pH 4.65). Both total and extractable nutrients were subsequently measured with an inductively-coupled plasma emission spectrometer (ARL 3580). For determination of exchangeable acidity, soil samples (7.5 g humus or 17 g mineral soil) were extracted with 150 ml 1 M KCl and titrated with 50 mM NaOH. Effective cation exchange capacity (ECEC) was expressed as the sum of charges of exchangeable Ca, Mg, K, Na, Al, Fe, Mn and H (cmol dm^{-3} fresh soil) and base saturation (BS) as the percentage of Ca, Mg, K and Na of ECEC.

2.4. Determination of microbial biomass N with fumigation–extraction (FE)

The fumigation–extraction (Brookes et al., 1985) was performed with the modifications described by Priha and Smolander (1997). Three replicates of 3 g d.m. humus or 6 g d.m. mineral soil samples, with the soil moisture content adjusted to 60% of the water-holding capacity (WHC), were used. Results were otherwise shown without the use of conversion factors, but for the evaluation of how big a proportion microbial biomass N is of total soil N, the flushes were converted to microbial biomass N by the formula of k μg $N_{mic} = (1.86 \times FE-N + 74.82)\,\mu g\ g^{-1}$ d.m. (Martikainen and Palojärvi, 1990) for the humus samples and $N_{mic} = (FE-N/0.54)$ (Brookes et al., 1985) for the mineral soil samples.

2.5. Incubation experiments to study N transformations

Net ammonification and nitrification were measured by incubating three replicates of 3 g d.m. humus or 6 g d.m. mineral soil samples, with the soil moisture content adjusted to 60% of the WHC, at 14°C for 40 d. The method used is described in detail in Priha and Smolander (1997).

The effect of pH on nitrification of the humus soils was studied with the soil suspension technique described by De Boer et al. (1992). The suspensions were made of 8 g d.m. humus samples and 350 ml mineral solution containing KH_2PO_4, $CaCl_2 \times 2H_2O$ and $MgSO_4 \times 6H_2O$ (0.2 mM each) and $(NH_4)_2SO_4$ (2.5 mM). Suspensions were incubated aerobically in 500-ml Erlenmeyer flasks with continuous shaking on a rotary shaker at room temperature (22°C) for 3 weeks. The flasks were kept in the dark and covered with foil caps. The pH of the suspensions was either maintained at the natural pH of the soils, or adjusted to pH 6 with Na_2CO_3; duplicate flasks for both treatments were done. During the incubation, pH was measured daily, and adjusted when necessary. Samples (50 ml) were taken at the beginning of the experiment and weekly after that. To ensure the availability of ammonium 1 ml of a 250 mM $(NH_4)_2SO_4$ solution was added weekly. Samples were filtered through Schleicher and Schuell 589[3] filter papers and NH_4^+-N and $(NO_2^- + NO_3^-)$-N concentrations were measured using a flow injection analyzer (FIAstar 5012 analyzer + 5042 detector, Tecator).

2.6. Enumeration of autotrophic nitrifiers

The numbers of autotrophic nitrifiers (ammonium and nitrite oxidizers) were estimated by the most probable number (MPN) method. Fresh soil samples (20 g) were mixed with 180 ml of sterilized H_2O in an Omni mixer (OCI Instruments) for 1 min at half speed. Tenfold dilutions of the suspensions were made in sterilized H_2O by using 18 ml dilution blanks and MPN tubes containing 3 ml of medium were inoculated with 1 ml of the diluted suspensions. The modified media of Bhuyia and Walker (1977) were used (Martikainen, 1985). In the NH_4 and NO_2 media the pH was 7.5, and concentrations of $(NH_4)_2SO_4$ and $NaNO_2$ were 0.1 g l^{-1}. Five replicates of each dilution were made for both ammonium and nitrite oxidizers. Inoculated MPN-tubes and control tubes were incubated at room temperature (22°C) in the dark for 10 weeks. At the end of the incubation, production of NO_2^- by ammonium oxidizers was checked with diphenylamine indicator (Rowe et al., 1977) and production of NO_3^- by nitrite oxidizers with Griess–Ilosway reagent (Alexander and Clark, 1965).

2.7. Measurement of denitrification and denitrification enzyme activity (DEA)

For measurement of denitrification, triplicate soil samples (3 g d.m. humus samples or 6 g d.m. mineral soil samples) with soil moisture content adjusted to 100% of the WHC were incubated at 14°C in 125 ml glass bottles under 10 kPa partial pressure of acetylene. The potential denitrification activity was determined by adding 20 μg KNO_3-N g^{-1} d.m. soil. The N_2O evolved was measured after 1 and 2 d incubations with a gas chromatograph (Hewlett Packard 6890 Series), equipped with an ECD and a Megapore GS-Q column (J&W Scientific), 30 m in length, using He (10 ml min^{-1}) as carrier gas and $ArCH_4$ (95:5) as the make-up gas. The temperatures of the detector, injector and oven were 300, 100 and 30°C, respectively. Results given are production rates of N_2O-N between 1 and 2 d. The solubility of N_2O in water was taken into account in the calculations (Moraghan and Buresh, 1977).

Denitrification enzyme activity (DEA) aims at determining the activity of preexisting denitrifying enzymes in soil, without allowing denitrifying organisms to proliferate (Luo et al., 1996). DEA was measured with three replicates and the same amounts of soil as above, but adding solutions of KNO_3 and glucose to give a NO_3-N concentration of 50 μg ml^{-1} soil water, a glucose concentration of 15 mg ml^{-1} soil water (found to be optimal in preliminary experiments) and a soil moisture content of 100% of the WHC. The bottles were evacuated and flushed with pure N_2. Headspace gas (12 ml) was removed from the bottles and replaced with C_2H_2 to give a partial pressure of 10 kPa. The samples were incubated for approximately 5 h in the dark at 22°C and N_2O produced was measured as described above.

2.8. Determination of acetylene reduction (nitrogenase activity)

Acetylene reduction was measured as described in Priha and Smolander (1997). Briefly, triplicate soil samples were incubated at 14°C under ambient air or 10 kPa partial pressure of acetylene. The potential nitrogenase activity was determined by adding a glucose–water solution. The ethylene evolved was measured after 1 and 2 d incubations with a GC (Varian 3600).

2.9. Correlations between microbiological and chemical soil characteristics

To evaluate the relationships between microbiological and chemical factors, Pearson correlations were calculated (Ranta et al., 1989).

3. Results

In order to be able to compare these very different soils, all results are given per fresh soil volume. The differences between sites, tree species and depths were, however, almost exactly the same as when the results were calculated g^{-1} soil organic matter.

3.1. Physical and chemical characteristics of the soils

At both sites, the humus layer was thickest under spruce and thinnest under birch (Table 2). The soil pH(H_2O) varied from 3.8 to 5.0 and was lowest in spruce soil at both sites in all soil layers. In the humus layer of both sites the pH was about 0.5 units higher under birch than under pine, but in the mineral soil the pH under pine and birch was roughly the same. The soil organic matter content and content of total C were variable, and there was no clear tree species effect. At the OMT-site, the soil organic matter content, on a soil volume basis, was not reduced in the mineral soil layers as much as ~~in~~ at the VT-site. The content of total N was highest in pine soil and lowest in spruce soil at the OMT-site, except in the deepest layer. At the VT-site the content of total N was highest in birch soil, except in the deepest layer. The C-to-N ratios were variable, but in the humus layers of both sites the C-to-N ratio was lowest under birch. C-to-N ratios were higher ~~in~~ at the VT- than ~~in~~ at the OMT-site.

The contents of total P, K, Ca and Mg were variable, and there were no consistent tree species trends, except that the content of Ca at the VT-site was twofold higher in birch than in pine or spruce soil in all soil layers (Table 2).

The content of soluble P was highest in spruce soil in the humus layer of the OMT-site and in birch soil in the humus layer of the VT-site (Table 2). In the mineral soil layers the contents were roughly the same. The effective cation exchange capacity (ECEC) was highest in humus layers under birch, especially at the VT-site. In mineral soils of both sites ECEC was lowest under birch. Base saturation did not vary much at the

Table 2
Some soil characteristics of the experimental sites. For explanation of site types, see Table 1

Site	Soil layer	Tree species	Average thickness of the humus layer	pH (H_2O)	Fresh bulk density ($g\ cm^{-3}$)	Organic matter ($mg\ cm^{-3}$ soil)	Total organic C ($mg\ cm^{-3}$ soil)	C-to-N ratio	Total nutrients obtained with dry combustion and HCl-extraction ($mg\ cm^{-3}$ soil)					Soluble P ($\mu g\ cm^{-3}$ soil)	ECEC (cmol dm^{-3})	BS(%)
									N	P	K	Ca	Mg			
OMT	humus layer	pine	4	4.5	0.52	85	46	25	1.8	0.19	0.17	0.47	0.33	9.6	4.6	49
		spruce	5	4.2	0.36	82	37	26	1.4	0.13	0.09	0.55	0.11	19.0	3.7	73
		birch	2	5.0	0.58	72	33	22	1.5	0.20	0.23	0.70	0.46	8.3	5.0	68
	0–3 cm mineral soil	pine		4.4	0.75	76	40	19	2.1	0.30	0.25	0.37	0.57	4.4	5.4	25
		spruce		3.8	0.71	79	36	22	1.6	0.26	0.19	0.36	0.30	3.3	5.4	28
		birch		4.5	0.73	70	36	18	1.9	0.26	0.25	0.39	0.55	3.8	4.9	34
	3–6 cm mineral soil	pine		4.5	0.88	59	32	18	1.8	0.31	0.35	0.41	0.93	3.2	7.3	13
		spruce		3.8	0.91	71	40	22	1.8	0.30	0.29	0.29	0.68	2.9	7.8	12
		birch		4.5	0.84	62	32	~~[illegible]~~ 18	1.7	0.26	0.27	0.29	0.64	2.4	5.9	17
VT	h ~~H~~umus layer	pine	5	4.0	0.28	81	42	37	1.1	0.09	0.08	0.33	0.05	16.5	2.6	63
		spruce	7	3.8	0.29	84	42	32	1.3	0.11	0.09	0.33	0.05	19.1	2.8	55
		birch	3	4.6	0.33	84	43	25	1.7	0.14	0.12	0.66	0.12	32.6	4.1	86
	0–3 cm mineral soil	pine		4.2	0.88	~~[illegible]~~ 50	28	35	0.8	0.18	0.25	0.19	0.24	4.4	5.0	17
		spruce		3.9	0.79	52	28	34	0.8	0.15	0.19	0.12	0.16	3.1	4.6	13
		birch		4.1	0.71	63	37	30	1.2	0.17	0.21	0.33	0.18	5.3	3.6	44
	3–6 cm mineral soil	pine		4.5	0.99	37	19	25	0.8	0.22	0.37	0.16	0.53	3.6	6.3	7
		spruce		4.0	0.89	47	21	31	0.7	0.18	0.29	0.12	0.41	2.7	6.6	6
		birch		4.3	0.93	39	22	31	0.7	0.25	0.37	0.27	0.44	5.8	5.1	16

ECEC means effective cation exchange capacity and BS base saturation.

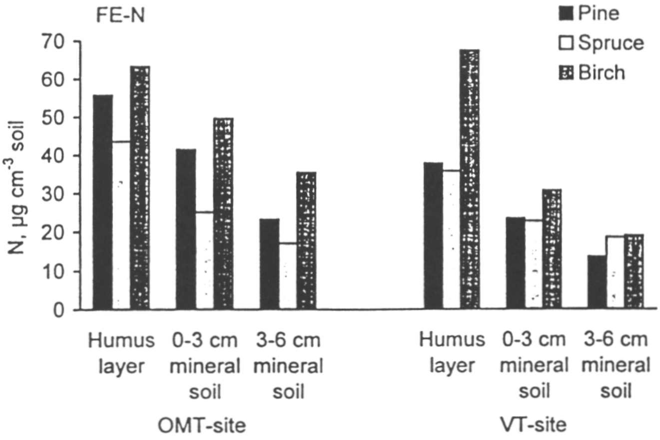


Fig. 1. The flush of extractable N from fumigation–extraction (FE) of the soils. The average coefficient of variation between three replicate soil samples was 6%. For explanation of site types, see Table 1.

OMT-site, except that it was lowest in pine soil in the humus layer. At the VT-site base saturation was highest under birch in all soil layers.

3.2. Microbial biomass N

The flush of N from fumigation–extraction was highest in birch soil and lowest in spruce soil in all depths at the OMT-site (Fig. 1). At the VT-site the flush was highest in birch soil, except in the 3–6 cm mineral soil layer. The flushes of N from fumigation varied from 240 to 880 ~~mg~~ g^{-1} organic matter. µg

The proportion of microbial biomass N of total soil N varied from 3 to 9%, and followed the flush of N from fumigation regarding tree species effects (results not shown). It was, on average, 7 and 4% of total soil N in the humus layer and in the mineral soil layers, respectively.

Table 3
NH_4^+-N and (NO_2^- + NO_3^-)-N concentrations, net ammonification, net nitrification and net formation of mineral N of the soils. The average coefficient of variation between three replicate soil samples was 8%. For explanation of site types see Table 1

Site	Soil layer	Tree species	Initial		Formation		
			NH_4^+-N (µg cm^{-3} soil)	(NO_2^- + NO_3^-)-N (µg cm^{-3} soil)	NH_4^+-N, (µg cm^{-3} soil 40 d^{-1})	(NO_2^- + NO_3^-)-N (µg cm^{-3} soil 40 d^{-1})	(NH_4^+ + NO_2^- + $NO3^-$)-N (µg cm^{-3} soil, 40 d^{-1})
OMT	humus layer	pine	25.4	1.7	−4.7	11.8	7.1
		spruce	29.7	0.0	15.4	0.2	15.6
		birch	22.4	0.0	−22.2	2.0	−20.2
	0–3 cm mineral soil	pine	15.9	3.6	−4.4	19.8	15.4
		spruce	11.9	0.0	9.7	0.0	9.7
		birch	22.4	0.0	−1.8	0.8	−1.0
	3–6 cm mineral soil	pine	10.1	2.5	0.2	7.7	7.9
		spruce	6.8	0.0	8.9	0.0	8.9
		birch	17.1	0.1	−0.2	1.6	1.4
VT	humus layer	pine	13.3	0.0	−12.2	0.0	−12.2
		spruce	26.5	0.0	0.9	0.0	0.9
		birch	37.0	0.0	28.5	0.0	28.5
	0–3 cm mineral soil	pine	5.2	0.0	−4.8	0.0	−4.8
		spruce	10.5	0.0	−10.1	0.0	−10.1
		birch	17.5	0.0	3.6	0.0	3.6
	3–6 cm mineral soil	pine	2.1	0.0	−1.8	0.0	−1.8
		spruce	6.3	0.0	−5.9	0.0	−5.9
		birch	6.3	0.0	−2.8	0.0	−2.8

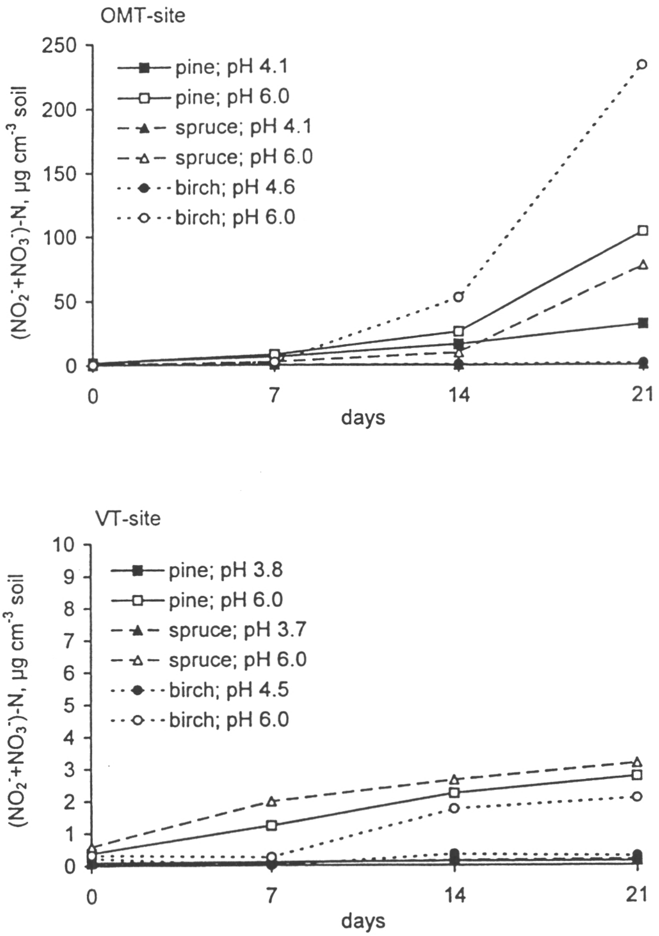


Fig. 2. Cumulative nitrate production in aerobic suspensions made of humus soil and mineral solution containing 2.5 mM $(NH_4)_2SO_4$. The average coefficient of variation between two replicate soil samples was 13%. For explanation of site types, see Table 1.

3.3. Ammonification and nitrification

All soils contained mineral N, birch soils more than the others except in the humus layer of the OMT-site and the 3–6 cm mineral soil at the VT-site (Table 3). The concentrations of mineral N varied from 60 to 450λg^{-1} organic matter. NO_3^--N was found only in µg pine soil from the OMT-site. Net formation of mineral N varied under different tree species and depths and was negative in many soils; more N was immobilized than mineralized. Net production of NO_3^--N occurred only at the OMT-site, and in considerable amounts only under pine. The nature of nitrification was confirmed to be autotrophic, because exposure to acetylene (3 Pa or 10 kPa partial pressure) totally inhibited it (results not shown).

Nitrate production by ammonium-enriched suspensions of the humus layer of the sites is shown in Fig. 2. In the suspensions from the OMT-site, only pine humus showed nitrification activity during the 3-week incubation in the original pH of the soil suspension, which was 4.1 for pine and spruce and 4.6 for birch. In suspensions where the pH was raised to 6, all the soils produced nitrate, birch soil more than the others, and pine soil more than at pH 4.1. In the suspensions from the VT-site nitrification activity was detected only when the pH was raised to 6, and the production was negligible compared to the OMT-site.

The variability of the MPN method was extremely high, and the results must therefore be viewed with caution (Fig. 3). Ammonium-oxidizers were most abundant in birch soil from the OMT-site (Fig. 3a). At

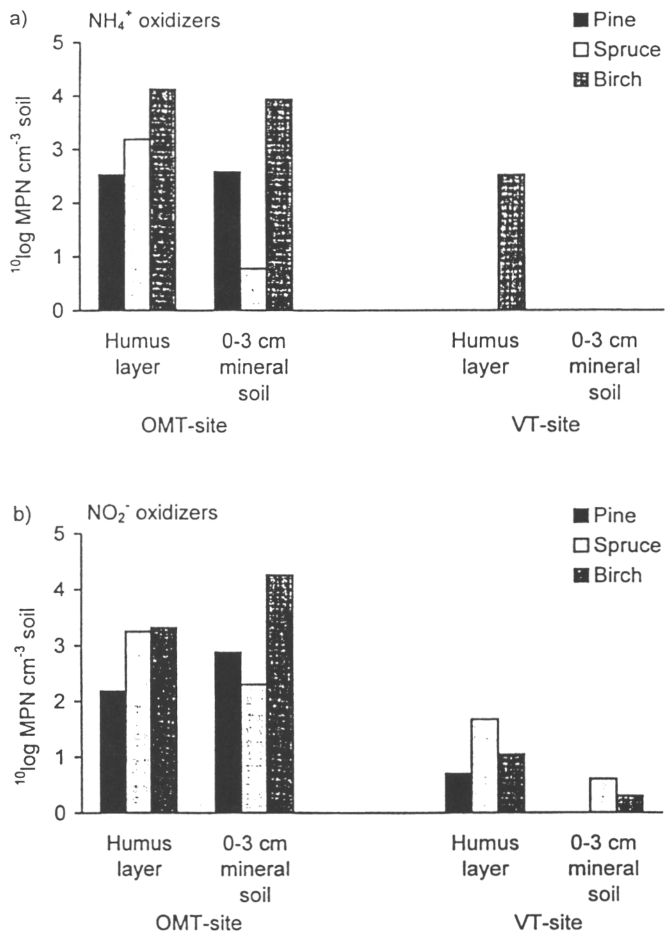


Fig. 3. Numbers of (a) ammonium- and (b) nitrite-oxidizers in the soils, measured by the most-probable-number (MPN) method. The average coefficient of variation between two replicate soil samples was 60%. For explanation of site types, see Table 1.

the VT-site ammonium-oxidizers were found only in the humus layer of birch. The numbers of nitrite-oxidizers tended to follow those of ammonium-oxidizers at the OMT-site (Fig. 3b). At the VT-site, small numbers of nitrite-oxidizers were found in all soils, except for the pine mineral soil.

3.4. Denitrification

Under 10 kPa partial pressure of acetylene, N_2O was produced at the OMT-site in pine soil in all soil layers and in birch soil in 3–6 cm mineral soil (Fig. 4a). When NO_3^-N was added, N_2O production started in birch soil in all soil layers and in spruce soil in all except the deepest layer (Fig. 4b). The production in spruce soil was considerably lower than in pine and birch soil. At the VT-site, N_2O was produced only when NO_3-N was added, and the production was very low except in the birch humus layer.

Denitrification enzyme activity (DEA) was 2–3 times lower in spruce soil than in pine and birch soils at the OMT-site (Fig. 5). At the VT-site the DEA was very low in all soils.

3.5. Acetylene reduction (nitrogenase activity)

None of the soils showed acetylene reduction activity (results not shown).

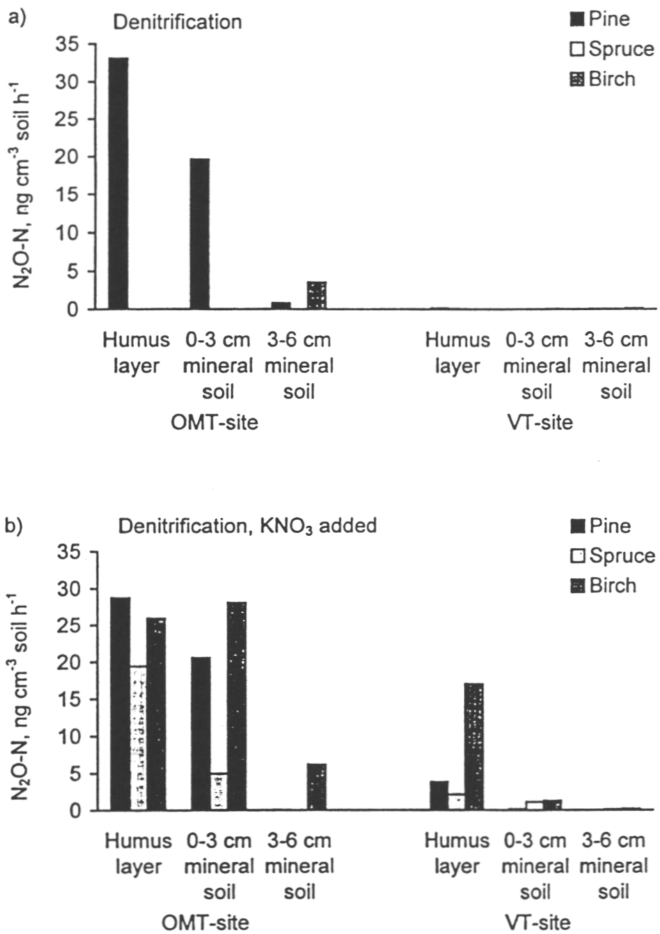


Fig. 4. Denitrification potential, measured as N_2O-N production under 10 kPa partial pressure of acetylene, (a) without substrate addition and (b) with 20 μg KNO_3-N g^{-1} d.m. soil added. The average coefficient of variation between three replicate soil samples was 15%. For explanation of site types, see Table 1.

3.6. Correlations between microbiological and chemical soil characteristics

The correlation coefficients for some chemical and microbiological variables are shown in Table 4. The flush of N from fumigation–extraction, the content of mineral N in soil, and denitrification potential with added KNO_3 all showed a significant ($P \leq 0.01$) positive correlation with the contents of total organic C, total N and base saturation. The numbers of ammonium oxidizers correlated positively with the soil pH and both ammonium and nitrite oxidizers correlated positively with total N and negatively with the C-to-N ratio.

4. Discussion

Our aim was to determine whether any changes in the soil microbial characteristics related to N transformations had occurred in soils of pine, spruce and birch in a period of about 60 yr. A weakness of this study was that there were no replicate plots of the tree species in the field. Both sites had, however, probably been originally homogeneous, so any differences which now appeared between plots are likely to be caused by the direct or indirect effects of these tree species. Thus, the major trends can be used to draw conclusions.

Soil microbes contained the highest amount of N in birch soil at both sites (Fig. 1). At the OMT-site mi-

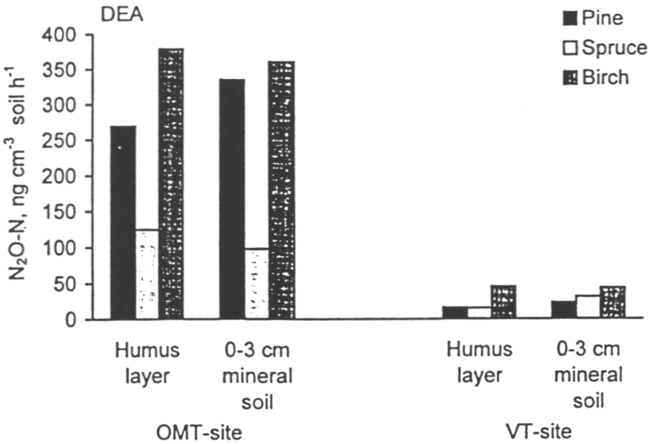


Fig. 5. Denitrification enzyme activity (DEA) of the soil samples. The average coefficient of variation between three replicate soil samples was 9%. For explanation of site types, see Table 1.

crobes contained more N in pine than in spruce soil, but at the VT-site pine and spruce were on the same level. Microbial biomass C in these soils behaved in a similar way (Priha et al., unpublished results). The soil pH and base saturation were highest in birch soil, which points to conditions in birch soil being more favorable to microbes than in pine and especially spruce soils because of higher availability of nutrients (Table 2). Trees affect the nutrient supply of soils both by their litter and root activities. Although the amount and composition of the above-ground litter of a certain tree species may vary greatly, birch leaf litter usually has a higher concentration of easily leached water-soluble compounds than spruce and pine needle litter, which makes birch leaf litter easier for microbes to decompose (Nykvist, 1963; Johansson, 1995). Pine needle litter often has less nutrients than that of spruce, but the high lignin concentration of spruce needle litter makes it more difficult to decompose (Johansson, 1995). The root activities of these tree species are not as well characterized as their litters, but at least birch and pine seedlings were shown to have more roots than spruce and also increase C mineralization and microbial biomass in the soil (Priha et al., 1998 ~~unpublished results~~). In addition to these direct effects of tree species on soil, the ground vegetation which establishes under a certain tree species affects soil properties. In these sites ground vegetation was different under different tree species, consisting of herbs and dwarf shrubs under pine, of mosses and dwarf shrubs under spruce, and of grasses and herbs under birch.

Net formation of mineral N in the incubation experiment was very variable and in several soils more N was immobilized than produced, which was surprising (Table 3). It is not likely that denitrification had been occurring in the incubation, because the denitrification potential of the majority of soils even at a higher soil moisture was negligible (Fig. 4a). Thus, there are two explanations for the negative net formation of N in the incubation. Firstly, there may have been intensive

Table 4
Pearson correlation coefficients of some chemical and microbiological soil variables. Bold font are significant ($p \leq 0.01$) correlations. $n = 18$ (FE-N, mineral N, net formation of mineral N, N_2O production and N_2O production with added KNO) or $n = 12$ (DEA, numbers of NH_4^+ and NO_2^--oxidizers). For explanation of site types, see Table 1

~~Site~~	pH(H_2O)	Organic C	Total N	C-to-N ratio	Base saturation
FE-N	0.49	**0.71**	**0.62**	−0.23	**0.86**
Mineral N	0.23	**0.80**	**0.70**	−0.25	**0.83**
Net formation of mineral N	−0.23	0.29	0.57	−0.28	0.01
N_2O production	0.28	0.33	0.44	−0.34	0.06
N_2O production with added KNO_3	0.49	**0.59**	**0.64**	−0.39	**0.73**
DEA	0.23	0.26	**0.72**	**−0.78**	0.18
Numbers of NH_4^+ oxidizers	**0.82**	0.20	**0.71**	**−0.81**	0.40
Numbers of NO_2^- oxidizers	0.46	0.20	**0.74**	**−0.84**	0.26

FE-N is the flush of N from fumigation and DEA the denitrification enzyme activity.

microbial immobilization in the incubation. It has been shown with ^{15}N studies that net and gross rates of N mineralization and nitrification do not necessarily correlate with each other, and rapid microbial assimilation of N has been suggested to be the main reason (Hart et al., 1994; Stark and Hart, 1997). The microbial immobilization of N in these soils was relatively high compared to other Finnish forest soils (e.g. Martikainen and Palojärvi, 1990), as microbial biomass N was, at its highest, 9% of total soil N. Secondly, tree roots may have stimulated N mineralization in the field. Roots of trees have been shown both to increase (Fisher and Stone, 1969; Bradley and Fyles, 1995) and decrease (Parmelee et al., 1993; Bradley and Fyles, 1996; Bradley et al., 1997) N mineralization in soil, and the effect has been shown to vary depending on the soil properties (Parmelee et al., 1993; Bradley and Fyles, 1996). A better understanding of the N flux in these soils could possibly be obtained using ^{15}N labelling.

Autotrophic nitrification was previously thought to be totally inhibited by low pH (<4–5), and the nitrification which occurs in acid forest soils to happen in soil microsites with a higher pH or to be heterotrophic. At present, the existence of autotrophic nitrification in acid conditions, especially at sites with unusually high ammonium availability, has been confirmed (De Boer et al., 1990; Martikainen et al., 1993; Persson and Wirén, 1995). Incubating soils in aerobic suspensions, where no microsites with a higher pH can occur, allows the determination of the true pH-dependency of the nitrifier communities. In soil suspension studies of Finnish fertilized soils nitrification showed a strict response to a pH gradient from 4.4 to 6.2: higher pH values resulted in higher nitrate production (Paavolainen and Smolander, 1998). The only soil in our study, which produced nitrate in considerable amounts during aerobic incubation and in soil suspension at the natural soil pH, was the pine soil from the OMT-site (Table 3, Fig. 2). The nitrifier community in the pine soil from the OMT-site was thus more acid-tolerant than the nitrifier communities in the other soils studied.

When the pH in the soil suspension slurries was raised to 6, all soils from the OMT-site produced nitrate; birch humus layer more than the others (Fig. 2). The number of ammonium oxidizers was also highest in birch humus layer (Fig. 3). Numbers of both ammonium and nitrite oxidizers correlated positively with total soil N, and negatively with C-to-N ratio (Table 4). Nitrification potentials and to some extent the nitrification rates have been found to be related to the C-to-N ratio of the forest floor, with 25–27 as a critical ratio (Gundersen et al., 1998). The low nitrification activity in soil suspension experiment and low numbers of nitrifiers at the VT-site can thus be due to the higher C-to-N ratio compared to the OMT-site. Differences in C-to-N ratio, however, do not clearly explain differences in nitrification activity between different tree species at the OMT-site.

Both the soil suspension slurry at a higher pH and enumerating nitrifiers with the MPN-method measure the nitrification potential of the soil, as the amount of ammonium is not inhibiting nitrification. There was an agreement at the OMT-site between results from high pH soil suspensions and enumeration of ammonium oxidizers, while birch humus from the VT-site had a high number of ammonium oxidizers but did not produce any more nitrate in the soil suspension than pine and spruce humus. The usual neutral pH of the MPN-media can be inhibitory to acid tolerant nitrifiers, but should, however, be suitable for the acid-sensitive or acid-tolerant but pH-dependent nitrifiers in our soils (De Boer et al., 1989, 1990). The formation of aggregates by nitrifying bacteria, however, can distort the numbers obtained with the MPN counts. Thus, soil suspension slurry is probably a more reliable method for measuring the nitrification potential of a certain soil.

Denitrification in forest soil can be limited by lack of nitrate, low pH, low moisture and thus high pO_2, low temperature, or lack of a C source (e.g. Federer and Klemedtsson, 1988; Willison and Anderson, 1991; Henrich and Haselwandter, 1991, 1997). Lots of variability exists in the conditions used for measurement of denitrification potential and denitrification enzyme activity, which makes comparisons between different studies difficult. Denitrification enzyme activity measurements aim at determining the activity of preexisting denitrifying enzymes in soil, without allowing denitrifying organisms to proliferate, whereas denitrification potential measurements allow new enzymes to be synthesized (Federer and Klemedtsson, 1988; Luo et al., 1996). In our study, the denitrification potential measurements with and without added nitrate showed that denitrification was mainly limited by lack of nitrate in spruce and birch soils (Fig. 4). Nevertheless, also other factors played a role, because denitrification potential with added nitrate was lower in spruce soil than in pine and birch soil at the OMT-site, and very low in all soils of the VT-site. Denitrification potential with added nitrate correlated positively with total organic C and N, and base saturation of the soil, and DEA correlated positively with total N and negatively with the C-to-N ratio (Table 4). As discussed earlier, the content of total N was lower and the C-to-N ratio higher at the VT-site than at the OMT-site (Table 2). In addition to low nitrification activity, this could influence the lower denitrification potential at the VT-site.

The effect of tree species on soil is a slow process, and a long time is probably needed for trees to cause major changes. In 23–24-yr-old field afforestation sites there were no major differences in the chemical and microbial characteristics of soils under pine, spruce and birch (Priha and Smolander, 1997), in contrast to these 60-yr-old sites. At these older sites the soil pH, microbial biomass N, denitrification potential and denitrification enzyme activity tended to be lowest under spruce and highest under birch. At the fertile site, these differences were seen both in the humus layer and the mineral soil layers, but at the less fertile site the differences were obvious only in the humus layer. Different populations of nitrifiers existed in the soils, regarding numbers, activity and pH-dependency. Only the nitrifier community in pine humus layer from the fertile site was adapted to more acidic conditions. Higher C-to-N ratios probably explained the negligible nitrification activity and numbers of nitrifiers at the less fertile site.

Acknowledgements

We are grateful for Anneli Rautiainen for her able laboratory assistance. Laura Paavolainen is thanked for comments on the manuscript and Donald Smart for revising the language. Jaakko Rokkonen and Antero Mikkola provided important information of the sites. The Academy of Finland supported this work financially.

References

Alexander, M., Clark, F. E., 1965. Nitrifying bacteria. In: Black, C.A. (Ed.), Methods of Soil Analysis. Part 2. American Society of Agronomy, Madison, WI, pp. 1477–1483.

Beuker, E., 1994. Long-term effects of temperature on the wood production of *Pinus sylvestris* L. and *Picea abies* (L.) Karst. in old provenance experiments. Scandinavian Journal of Forest Research 9, 34–45.

Bhuyia, Z.H., Walker, N., 1977. Autotrophic nitrifying bacteria in acid tea soils from Bangladesh and Sri Lanka. Journal of Applied Bacteriology 42, 253–257.

Bradley, R.L., Fyles, J.W., 1995. Growth of paper birch (*Betula papyrifera*) seedlings increases soil available C and microbial acquisition of soil-nutrients. Soil Biology & Biochemistry 27, 1565–1571.

Bradley, R.L., Fyles, J.W., 1996. Interactions between tree seedling roots and humus forms in the control of soil C and N cycling. Biology and Fertility of Soils 23, 70–79.

Bradley, R.L., Titus, B.D., Fyles, J.W., 1997. Nitrogen acquisition and competitive ability of *Kalmia angustifolia* L., paper birch (*Betula papyrifera* Marsh.) and black spruce (*Picea mariana* (Mill.) B.S.P.) seedlings grown on different humus forms. Plant and Soil 195, 209–220.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology & Biochemistry 17, 837–842.

Cajander, A.K., 1949. Forest types and their significance. Acta Forestalia Fennica 56, 1–71.

Climatological Statistics in Finland 1961–1990, 1991. In: Meteorological Yearbook of Finland, vol. 90, part 1–1990, Suppl. The Finnish Meteorological Institute, Helsinki.

De Boer, W., Klein Gunnewiek, P.J.A., Troelstra, S.R., Laanbroek, H.J., 1989. Two types of chemolithotrophic nitrification in acid heathland humus. Plant and Soil 119, 229–235.

De Boer, W., Klein Gunnewiek, P.J.A., Troelstra, S.R., 1990. Nitrification in Dutch heathland soils. II. Characteristics of nitrate production. Plant and Soil 127, 193–200.

De Boer, W., Tietema, A., Klein Gunnewiek, P.J.A., Laanbroek, H.J., 1992. The chemolithotrophic ammonium-oxidizing community in a nitrogen-saturated acid forest soil in relation to pH-dependent nitrifying activity. Soil Biology & Biochemistry 24, 229–234.

Federer, C.A., Klemedtsson, L., 1988. Some factors limiting denitrification in slurries of acid forest soils. Scandinavian Journal of Forest Research 3, 425–435.

Finnish Statistical Yearbook of Forestry, 1997. Finnish Forest Research Institute. Gummerus Kirjapaino Oy, Jyväskylä, 348 pp.

Fisher, R.F., Stone, E.L., 1969. Increased availability of nitrogen and phosphorus in the root zone of conifers. Soil Science Society of America Proceedings 33, 955–961.

Gundersen, P., Emmett, B.A., Kjønaas, O.J., Koopmans, C.J., Tietema, A., 1998. Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. Forest Ecology and Management 101, 37–55.

Hart, S.C., Nason, G.E., Myrold, D.D., Perry, D.A., 1994. Dynamics of gross nitrogen transformations in an old-growth forest: the carbon connection. Ecology 75, 880–891.

Henrich, M., Haselwandter, K., 1991. Denitrifying potential and enzyme activity in a Norway spruce forest. Forest Ecology and Management 44, 63–68.

Henrich, M., Haselwandter, K., 1997. Denitrification and gaseous nitrogen losses from an acid spruce forest soil. Soil Biology and Biochemistry 29, 1529–1537.

Johansson, M.-B., 1995. The chemical composition of needle and leaf litter from Scots pine, Norway spruce and white birch in Scandinavian forests. Forestry 68, 49–62.

Luo, J., White, R.E., Ball, P.R., Tillman, R.W., 1996. Measuring denitrification activity in soils under pasture: optimizing conditions for the short-term denitrification enzyme assay and effects of soil storage on denitrification activity. Soil Biology & Biochemistry 28, 409–417.

Martikainen, P.J., 1985. Numbers of autotrophic nitrifiers and nitrification in fertilized forest soil. Soil Biology & Biochemistry 17, 245–248.

Martikainen, P.J., Palojärvi, A., 1990. Evaluation of the fumigation–extraction method for the determination of microbial C and N in a range of forest soils. Soil Biology & Biochemistry 22, 797–802.

Martikainen, P.J., Lehtonen, M., Lång, K., De Boer, W., Ferm, A., 1993. Nitrification and nitrous oxide production potentials in aerobic soil samples from the soil profile of a Finnish coniferous forest site receiving high ammonium deposition. FEMS Microbiology Ecology 13, 113–122.

Moraghan, J.T., Buresh, R., 1977. Correction for dissolved nitrous oxide in nitrogen studies. Soil Science Society America Journal 41, 1201–1202.

Muys, B., Lust, N., 1992. Inventory of the earthworm communities and the state of litter decomposition in the forests of Flanders, Belgium, and its implications for forest management. Soil Biology & Biochemistry 24, 1677–1681.

Nykvist, N., 1963. Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter. Studia Forestalia Suecica 3, 1–31.

Paavolainen, L., Smolander, A., 1998. Nitrification and denitrification in soil from a clear-cut Norway spruce (*Picea abies*) stand. Soil Biology & Biochemistry 30, 775–781.

Parmelee, R.W., Ehrenfeld, J.G., Tate, R.L., III, 1993. Effects of pine roots on microorganisms, fauna and nitrogen availability in two soil horizons of a coniferous forest spodosol. Biology and Fertility of Soils 15, 113–119.

Persson, T., Wirén, A., 1995. Nitrogen mineralization and potential nitrification at different depths in acid forest soils. Plant and Soil 168-169, 55–65.

Priha, O., Smolander, A., 1997. Microbial biomass and activity in soil and litter under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at originally similar field afforestation sites. Biology and Fertility of Soils 24, 45–51.

Ranta, E., Rita, H., Kouki, J., 1989. Biometria. 2nd ed. Yliopistopaino, Helsinki. 569 p.

Rowe, R., Todd, R., Waide, J., 1977. Microtechnique for most-probable-number analysis. Applied and Environmental Microbiology 33, 675–680.

Stark, J.M., Hart, S.C., 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385, 61–64.

Tamminen, P., 1993. Pituusboniteetin ennustaminen kasvupaikan ominaisuuksien avulla Etelä-Suomen kangasmetsissä. Summary: estimation of site index for Scot~~ch~~s pine and Norway spruce stands in South Finland using site properties. Folia Forestalia 819, 1–26.

Willison, T.W., Anderson, J.M., 1991. Denitrification potentials, controls and spatial patterns in a Norway spruce plantation. Forest Ecology and Management 44, 69–76.

Add:

Priha, O., Lehto, T., Smolander, A., 1998. Mycorrhizas and C and N transformations in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings. Plant and Soil, in press.

Paper III

Priha O., Grayston S.J., Hiukka R., Pennanen T. & Smolander A. Microbial community structure and characteristics of the organic matter in soils under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. Submitted manuscript.

III

Statement of the contribution of the individual authors in paper entitled

Microbial community structure and characteristics of the organic matter in soils under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites

by

Outti Priha, Susan J. Grayston*, Risto Hiukka, Taina Pennanen and Aino Smolander

Finnish Forest Reasearch Institute, P.O.Box 18, FIN-01301 Vantaa, Finland
*Macaulay Land Use Reseach Institute, Craigiebuckler, Aberdeen AB15 8QH, U.K.

Contributions of the authors listed are:

Outi Priha: Corresponding author. Performing experimental work, except for FTIR spectra. Responsible for writing and interpretation of the results.
Susan J. Grayston: Advice in performing the Biolog-measurements.
Risto Hiukka: Performing FTIR spectra analyses and interpreting them.
Taina Pennanen: Advice in performing phospholipid fatty acid analyses and interpreting them.
Aino Smolander: Supervisor of the Ph.D. studies of O. Priha.

All authors have given their comments of the paper before submission.

Outi Priha

Susan J. Grayston

Risto Hiukka

Taina Pennanen

Aino Smolander

MICROBIAL COMMUNITY STRUCTURE AND CHARACTERISTICS OF THE ORGANIC MATTER IN SOILS UNDER *PINUS SYLVESTRIS*, *PICEA ABIES* AND *BETULA PENDULA* AT TWO FOREST SITES

OUTI PRIHA, SUSAN J. GRAYSTON*, RISTO HIUKKA, TAINA PENNANEN and AINO SMOLANDER
Finnish Forest Research Institute, P.O.Box 18, FIN-01301 Vantaa, Finland
*Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen AB15 8QH, U.K.

Summary - Microbial biomass C (C_{mic}), C mineralization rate, phospholipid fatty acid (PLFA) profiles and community level physiological profiles (CLPPs) using Biolog were determined from the humus and mineral soil layers in adjacent stands of Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) Karst.) and silver birch (*Betula pendula* Roth) at two forest sites of different fertility. In addition, the Fourier-transform infrared (FTIR) spectra were run on the samples for characterization of the organic matter. C_{mic} and C mineralization rate tended to be lowest under spruce and highest under birch, at the fertile site in all soil layers and at the less fertile site in the humus layer. Microbial community structure was also different in soils under different tree species. In the humus layer the PLFAs separated all tree species and in the mineral soil spruce was distinct from pine and birch. The PLFA profiles and plate counts of soils suggested that birch stands favoured the growth of Gram-negative bacteria, whereas at pine and spruce stands Gram-positive species appeared to be more common. CLPPs did not distinguish microbial communities from the different tree species in the humus layer, but tended to separate birch from the conifers in the mineral soil. The FTIR spectra did not separate the tree species, but clearly separated the two sites.

INTRODUCTION

Plant cover and especially the dominant tree species affect the biological and chemical fertility of the soil. Although there are not many studies on the effects of different tree species on soil microbes, microbial biomass, activity and community structure have all been shown to be affected by different tree species (Turner *et al.,* 1993; Bauhus *et al.,* 1998; Grayston and Campbell, 1996; Grayston *et al.*, 1996). Tree species can also affect the chemical characteristics of soil (Binkley and Valentine, 1991; Howard *et al.,* 1998). In a previous study it was shown that soil pH, nutrient content and N transformations differed in adjacent stands of Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) Karst.) and silver birch (*Betula pendula* Roth) at two sites of contrasting fertility (Priha and Smolander, 1999). The soil pH, microbial biomass N, and denitrification potential were lowest under spruce and highest under birch. Also numbers of nitrifiers and their activity and pH-dependency varied under these tree species.

The aim of this study was to determine microbial community structure and characteristics of the organic matter under pine, spruce and birch at the two forest sites used in the previous study (Priha and Smolander, 1999).

Microbial biomass C (C_{mic}), C mineralization, plate counts of culturable microorganisms, microbial phospholipid fatty acid (PLFA) composition, community level physiological profiles (CLPPs) using Biolog plates, and organic matter characteristics (infrared spectra) were determined.

MATERIALS AND METHODS

Study sites and sampling

Two field experiments were studied, one at Punkaharju in South-East Finland (61°48' N, 29°18' E), and one at Uurainen in Middle-Finland (62°25' N, 25°29' E). Both experiments contained one plot of each tree species (pine, spruce and birch) adjacent to each other, the average size of a plot being 0.5 ha. The Punkaharju experiment was established in 1931 and is a fertile site, classified as an *Oxalis acetocella - Vaccinium myrtillus* type (OMT) (Cajander, 1949; Beuker, 1994). The Uurainen experiment was established in 1936-40, and is less fertile than Punkaharju, classified as a *Vaccinium vitis-idaea* type (VT) (Cajander, 1949; Jaakko Rokkonen, personal communication). At both sites, the soil type was podzol and the humus type mor. More detailed descriptions of the study sites and data of the physical and chemical characteristics of the soils are given in Priha and Smolander (1999).

Samples were taken in July 1995 for determination of C_{mic}, C mineralization and FTIR-spectra, and in September 1996 for PLFA, plate counts and CLPP analyses. Twenty soil cores were taken systematically from each plot (to 10 cm depth, core diameter 5.8 cm, 0.5-1 m from the nearest tree). Samples were divided into humus layer, 0-3 cm mineral soil layer and 3-6 cm mineral soil layer. The samples were combined in a depthwise manner, green plant material and coarse roots were removed, and the samples were sieved (mesh size 5.7 mm) and stored at +4°C or -18°C. The CLPPs and plate counts were made from samples kept at +4°C within one week and C_{mic} and C mineralization within one to two weeks from sieving. PLFAs and FTIR-spectra were measured from the soils kept at -18°C. Other analyses were done for all soil depths (humus, 0-3 cm and 3-6 cm mineral soil), but PLFAs, CLPPs and plate counts only for humus and 0-3 cm mineral soil samples.

Fumigation-extraction, substrate-induced respiration and basal respiration

C_{mic} with fumigation-extraction (FE) and substrate-induced respiration (SIR) were measured as described by Priha and Smolander (1997), based on the approaches of Vance *et al.* (1987) (FE-C) and Anderson and Domsch (1978) and West and Sparling (1986) (SIR). Results were otherwise shown without the use of conversion factors, but for the evaluation of how large a proportion C_{mic} is of total soil organic C, the flushes were converted to C_{mic} by the formula of C_{mic} = (FE-C / 0.35) (Sparling *et al.*, 1990). Basal respiration, which describes the C mineralization rate, was evaluated as CO_2-C production in a 48 h incubation at 14°C (Priha and Smolander 1997). For all these measurements, triplicate 2 g d.m. (dry matter) humus samples and 6 g d.m. mineral soil samples were used, with soil moisture adjusted to 60% of the water-holding capacity (WHC).

PLFA analysis

The phospholipid extraction and analysis of PLFAs was performed as described by Frostegård *et al.* (1993). Briefly, 0.5 g f.w. (fresh weight) humus and 1 g f.w. (OMT-site) or 2.5 g f.w. (VT-site) mineral soil samples were extracted with a chloroform:methanol:citrate buffer -mixture (1:2:0.8), and the lipids were separated into neutral lipids, glycolipids, and phospholipids in a silicic acid column. The phospholipids were subjected to mild alkaline methanolysis, and the fatty acid methyl esters were separated by gas chromatography (Hewlett Packard 5890) equipped with a flame ionization detector and a HP-5 (phenylmethyl silicone) capillary column, 50 m in length, using He as carrier gas. Peak areas were quantified by adding methyl nonadecanoate fatty acid (19:0) as an internal standard.

Fatty acids are designated in terms of total number of carbon atoms : number of double bonds, followed by the position of the double bond from the methyl end of the molecule. The prefixes a, i and br indicate anteiso, iso and unknown branching, respectively. The prefix cy indicates a cyclopropane fatty acid, and methyl branching (Me) is indicated as the position of the methyl group from the carboxyl end of the chain. The prefix C (C15:1) indicates that the PLFA has 15 carbon atoms and one double bond, but the arrangement of the carbon atoms (e.g. branching position) is not confirmed.

The sum of PLFAs considered to be mainly of bacterial origin (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) was chosen to represent bacterial biomass (bacterial PLFAs) (Frostegård and Bååth, 1993). The quantity of 18:2ω6,9 was used as an indicator of fungal biomass (fungal PLFA). The ratio fungal to bacterial PLFAs was also calculated.

CLPPs

Community level physiological profiles (CLPPs) were conducted using Biolog plates, according to Campbell *et al.* (1997). Briefly, to extract the microbes, triplicate soil samples (10 g f.w.) were shaken in 100 ml ¼ strength Ringers solution (Oxoid) for 10 min. The 10^{-4} dilution of each soil sample was centrifuged at 750 *g* for 10 min to remove soil and root particles which might introduce additional C into the wells. A 150 μl aliquot of the supernatant from the centrifuged samples was added to each well of a Biolog GN plate (Biolog Inc., Hayward, California, USA) and an MT plate with 30 additional carbon sources representing compounds reported in the literature to be plant root exudates (Campbell *et al.* 1997). Plates were incubated at 15°C and colour development measured as absorbance at 590 nm (A590) using microplate reader (Emax, Molecular Devices, Oxford, UK). Absorbance was measured first at 0 h, then every 24 h for 5 days, and then at 10 d and 15 d.

We compared all 125 C sources with the 61 identified as being plant root exudates (30 from MT plate plus 31 from GN plate; see Campbell *et al.*, 1997). When calculating the results, first the 0 h reading was subtracted from each of the wells, as some compounds were initially coloured. The blanks from both GN and MT plates were then subtracted and the average well colour development (AWCD; Garland and Mills, 1991) was calculated for each time point. In order to eliminate variation in AWCD, which may arise from different cell densities in different samples, Garland (1996) recommended comparison of samples of equivalent AWCD. We used on each sample the time point at which the AWCD was closest to the value of 0.75. This time point was 5 or 10 d for the humus samples and 10 or 15 d for the mineral soil samples. The values from different time points were then all divided by their respective AWCDs.

Plate counts

Triplicate soil samples (10 g f.w.) were shaken in 100 ml ¼ strength Ringers solution (Oxoid) for 10 min. The samples were then serially diluted to 10^{-7} in ¼ strength Ringers solution and suspensions (0.1 ml) spread, in duplicate, onto the following media: Tryptone Soy agar (1/10 Oxoid strength) plus cycloheximide (50 mg l^{-1}) for enumeration of bacteria and actinomycetes; *Pseudomonas* Isolation agar (Oxoid) selective for populations of pseudomonads; Czapek Dox agar (Oxoid) + streptomycin sulphate (50 mg l^{-1}) + tetracycline hydrochloride (50 mg l^{-1}) + ampicillin (10 mg l^{-1}) for enumeration of yeasts and fungi. The plates were incubated at 25°C and colonies counted after 5-6 days and again after 13-14 days.

FTIR spectra

The Fourier-transform infrared (FTIR) spectra of the mortared samples were measured with a Perkin Elmer System 2000 FTIR instrument using KBr disk technique. Samples were scanned 32 times with resolution 4 cm^{-1} using scan range from 4000 to 370 cm^{-1}.

Statistical analyses

The mole percents of the PLFA values, the Biolog values divided by AWCDs, and the FTIR spectra were subjected to principal component analysis (PCA) using a correlation matrix for PLFAs and Biolog and covariance matrix for FTIR spectra (Mustonen, 1995). PCA was done separately for humus and mineral soil samples. The Systat 6.0.1 (SPSS Inc, 1996) statistical software was used for PLFA and Biolog values and the Unscrambler 6.1 (CAMO AS, 1996) software for FTIR spectra.

RESULTS

Microbial biomass C and C mineralization rate

C_{mic} and C mineralization were affected by tree species, sites and in depthwise distribution. On an organic matter basis, the flush of C from fumigation and especially substrate-induced respiration were highest in birch soil, and lowest in spruce soil in all soil depths at the OMT-site (Table 1). At the VT-site both were highest in birch soil in the humus layer. C_{mic} was, on average, 2.3% of total soil organic C, and followed FE-C and SIR regarding tree species effect (results not shown). Basal respiration was highest under birch at both sites in all soil layers except the 0-3 cm mineral soil layer at the VT-site (Table 1). The average coefficient of variation between three replicate soil samples was 6%. These tree species effects were the same when results were calculated per soil volume.

Table 1. The flush of C from fumigation-extraction (FE), CO_2-C production from basal and substrate-induced respiration (SIR), the total amount of microbial phospholipid fatty acids (PLFAs), ratio of fungal : bacterial PLFAs and ratio of *trans* unsaturated 16:1ω7 to *cis* unsaturated 16:1ω7. OMT = *Oxalis acetocella* - *Vaccinium myrtillus* -type and VT = *Vaccinium vitis-idaea* -type (Cajander 1949). Soil samples were taken in July 1995 for other analyses and in September 1996 for PLFA analysis.

Site	Soil layer	FE-C, mg g^{-1} o.m.			Basal respiration CO_2-C, µg g^{-1} o.m. h^{-1}			SIR CO_2-C, µg g^{-1} o.m. h^{-1}			Microbial PLFAs µmol g^{-1} o.m.			Fungal PLFA / bacterial PLFAs			16:1ω7t / 16:1ω7c		
		Pine	Spruce	Birch	Pine	Spruce	Birch	Pine	Spruce	Birch	Pine	Spruce	Birch	Pine	Spruce	Birch	Pine	Spruce	Birch
OMT	Humus layer	5.2	3.8	5.5	16	17	20	144	100	211	2.5	2.0	3.8	0.12	0.13	0.15	0.12	0.27	0.11
	0-3 cm mineral soil	4.5	2.9	5.1	6	7	12	89	58	153	3.5	2.1	3.3	0.05	0.08	0.11	0.18	0.35	0.16
	3-6 cm mineral soil	3.4	3.0	4.4	4	5	7	71	37	104									
VT	Humus layer	3.8	3.4	5.2	14	13	22	118	88	207	2.9	2.5	3.9	0.20	0.25	0.23	0.16	0.22	0.15
	0-3 cm mineral soil	4.3	4.2	3.5	10	11	10	92	80	96	3.0	2.6	2.7	0.12	0.12	0.14	0.20	0.26	0.17
	3-6 cm mineral soil	3.9	4.1	4.4	7	8	11	73	75	128									

o.m. = organic matter

Phospholipid fatty acid (PLFA) composition

The total amount of microbial PLFAs was highest in birch soil in the humus layers of both sites and lowest under spruce in all samples (Table 1). The scores of the first two principal components of the humus samples are plotted in Fig. 1a. Tree species separated along the first axis, which explained 36% of the variance. The two forest sites separated along the second axis, which explained 30% of the variance. In the mineral soil samples, the first axis, which explained 44% of the variance, separated spruce from pine and birch (Fig. 1b). As in humus samples, the two sites were separated along the second axis, which explained 31% of the variance. The loadings for the individual PLFAs for the first and second principal components for humus and mineral soil samples are shown in Fig 2. In humus samples PLFAs 18:1ω7 and 10Me18:0 were relatively more abundant under birch, 16:1ω9 under pine, and 16:1ω7t, i15:0 and a17:0 under spruce. In the mineral soil the PLFAs typical for the spruce samples were the same as in the humus layer, while 18:1ω7 and 18:1ω9 were more abundant in the birch and pine samples. In both soil layers the relative proportions of PLFAs i16:1 and 10Me16:0 were higher in the spruce and pine soil compared to the birch soil. The ratio of 16:1ω7t to 16:1ω7c was higher and the relative amount of 16:1ω5 lower in spruce samples compared to those of birch and pine samples in both soil layers.

There was more 18:2ω6,9, indicative of fungal biomass, and the ratio of fungal to bacterial PLFAs was higher in soils from the VT-site than from the OMT-site (Fig 2, Table 1), but 18:2ω6,9 did not have a great influence in separating the tree species.

CLPPs

The scores for the first two principal components of the CLPPs for humus samples are shown in Fig. 3a,b. There was no clear separation of the tree species either with all 125 C sources or with exudate C sources. The exudate C sources did separate the two sites from each other along PC axis 2, which explained 19% of the variation. In the mineral soils, birch soils had a tendency of separating from the others, both with all C sources and with exudate C sources (Fig. 3c,d)

Plate counts

There were tree species specific differences in numbers of culturable bacteria (Table 2). Birch soil had higher numbers of culturable bacteria in both soil layers of the OMT-site and in the humus layer of the VT-site. Numbers of pseudomonads were highest under birch and lowest under spruce in the mineral soil layer of the OMT-site and in the humus layer of the VT-site.

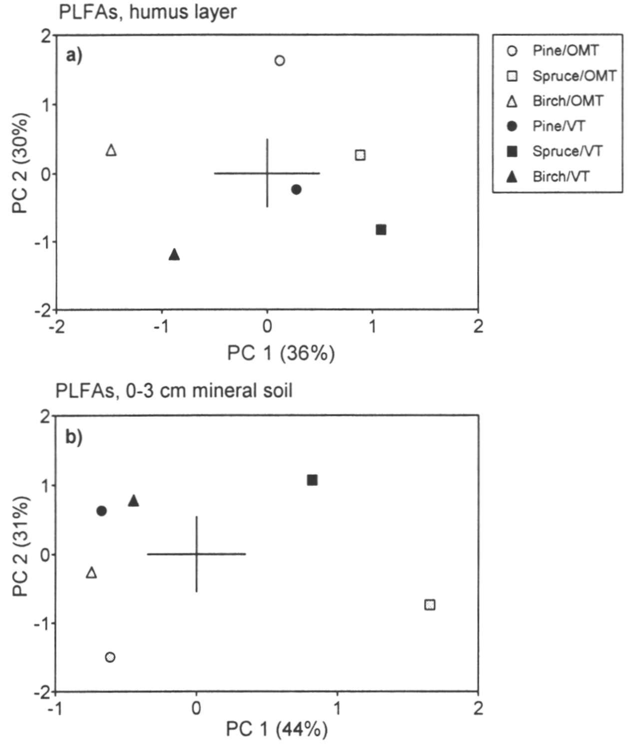


Fig. 1. The scores of the first two principal components of the PLFAs of the a) humus and b) mineral soil samples.

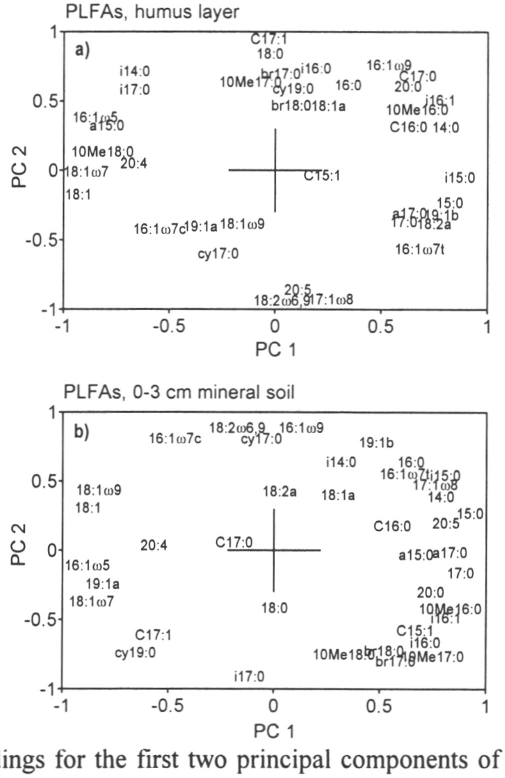


Fig. 2. The loadings for the first two principal components of the a) humus and b) mineral soil samples.

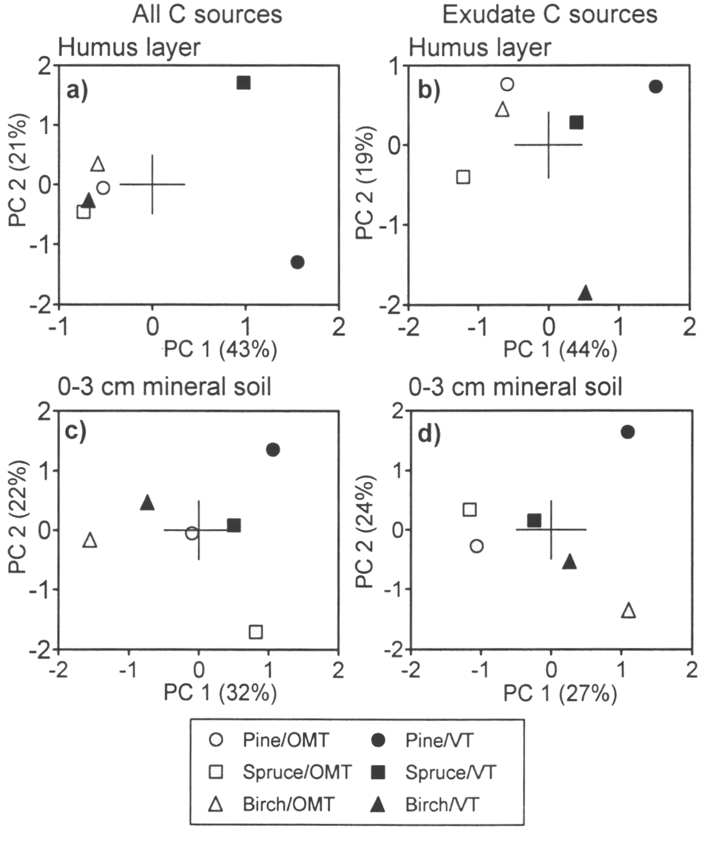


Fig. 3. The scores for the first two principal components of the CLPPs for a) humus samples using all 125 C sources, b) humus samples using 61 exudate C sources, c) mineral soil samples using all 125 C sources and d) mineral soil samples using 61 exudate C sources.

Organic matter characterization (FTIR spectra)

Examples of FTIR spectrum of one humus and one 0-3 cm mineral soil sample are shown in Fig. 4. The scores of the first two principal components of the humus samples are plotted in Fig. 5a. Tree species were not separated from each other. The two forest sites tended to separate along the first axis, which explained 80% of the variance. In the mineral soil samples, the first principal component, which explained 78% of the variance for 0-3 cm samples and 79% of the variance for 3-6 cm samples, was also the forest site axis (Fig. 5b, c). Again the tree species were not separated from each other.

Table 2. Results from plate counts of the soils. For explanation of site types, see Table 1.

Site	Soil layer	Bacteria, cfu g^{-1} o.m.			Pseudomonads, cfu g^{-1} o.m.			Fungi cfu g^{-1} o.m.			Yeasts cfu g^{-1} o.m. soil		
		Pine	**Spruce**	**Birch**	**Pine**	**Spruce**	**Birch**	**Pine**	**Spruce**	**Birch**	**Pine**	**Spruce**	**Birch**
OMT	Humus layer	1.2×10^8	3.1×10^7	3.2×10^8	2.3×10^6	3.2×10^6	7.3×10^6	1.6×10^6	5.2×10^6	3.4×10^6	1.3×10^5	1.3×10^6	1.3×10^6
	0-3 cm mineral soil	6.3×10^7	3.1×10^7	2.4×10^8	5.6×10^5	3.5×10^4	4.4×10^6	1.9×10^6	2.0×10^6	4.5×10^6	5.4×10^5	3.0×10^5	4.3×10^6
VT	Humus layer	8.7×10^7	1.8×10^8	1.4×10^9	2.4×10^6	3.7×10^5	2.3×10^7	2.4×10^6	1.4×10^6	6.7×10^6	6.6×10^5	1.3×10^5	2.0×10^6
	0-3 cm mineral soil	1.1×10^8	4.6×10^7	1.6×10^8	9.9×10^5	1.5×10^6	6.2×10^6	2.9×10^6	5.1×10^6	4.4×10^6	9.2×10^5	8.5×10^5	7.4×10^5

o.m. = organic matter
cfu = colony forming unit

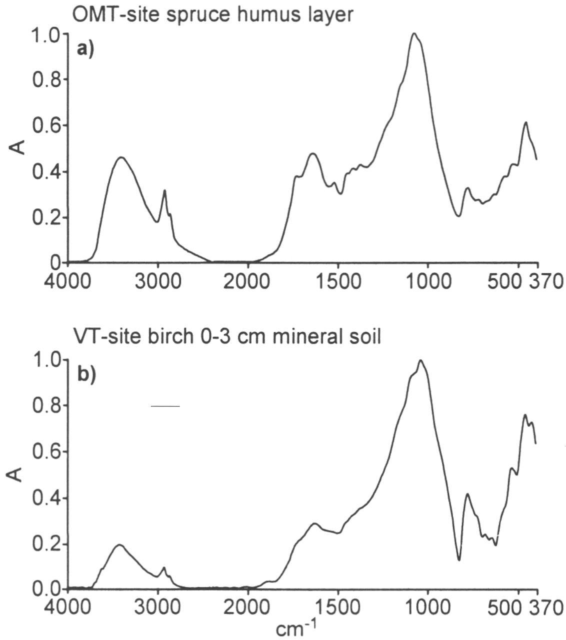


Fig. 4. Examples of infrared spectra. a) Spruce humus from the OMT-site and b) birch mineral soil from the VT-site.

The most dominating loadings for the first PC for humus samples were at wavelengths between 1100 and 1000 cm^{-1}, attributed to C-O stretching of carbohydrates, skeletal vibrations of aliphatic groups and Si-O stretching of silica. In the PCA model of the 0-3 cm mineral soil samples the dominating loading was 1620 cm^{-1}. The bands between 1660 and 1500 cm^{-1} are caused by several overlapping functional groups (different kind of C=C including conjugation with C=O, ungonjugated C=O, C=N, COO^- and N-H absorptions), thus definite interpretation cannot be given. In the PCA model of the 3-6 cm mineral soil samples dominating loadings were 1620 and 1085 cm^{-1} (Si-O stretching of silica). The interpretation of the spectra is based on data published by Stevenson and Goh (1971), Gerasimowicz *et al.* (1983), Senesi *et al.* (1987), Liu and Ryan (1997) and Lin-Vien *et al.* (1991).

DISCUSSION

The aim of this study was to characterize the soil microbial communities, their activity, and soil chemical characteristics under pine, spruce and birch at two forest sites of different fertility. A weakness of this study was that there were no replicate plots of the tree species in the field, and thus no real replicates in the analyses. Because of this, especially the results from PCA analyses must be regarded tentative. Nevertheless, as the effect of tree species on soil is a slow process, and long-term replicated field experiments, lasting the age of one tree generation, are lacking, these sites are worth studying.

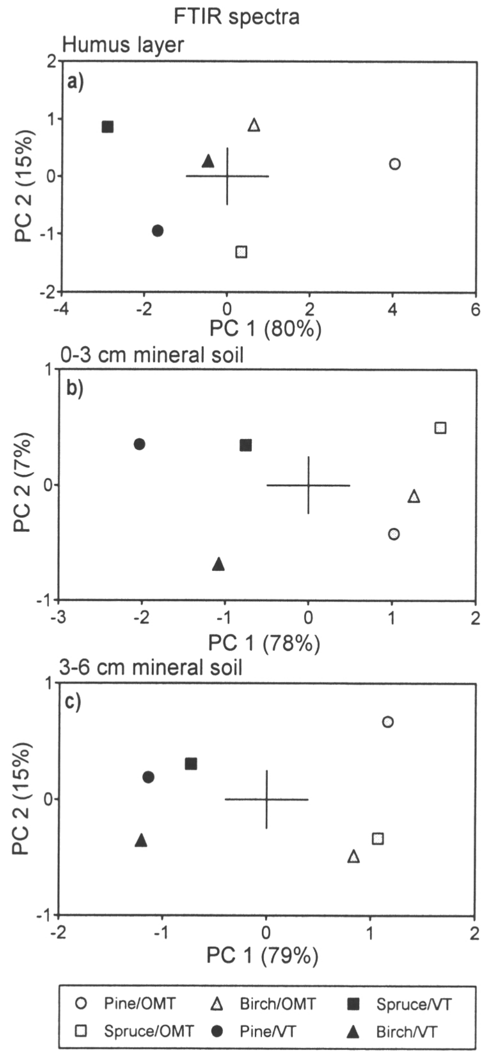


Fig. 5. The scores for the first two principal components of the FTIR spectra of a) humus samples, b) 0-3 cm mineral soil samples and c) 3-6 cm mineral soil samples.

Microbial biomass, measured by both FE and SIR, was highest under birch and lowest under spruce, and C mineralization rate was highest under birch (Table 1). Microbial biomass N followed these same trends, being greatest under birch and lowest under spruce (Priha and Smolander, 1999). These results were expressed on an organic matter basis, and thus reflect the quality of the organic matter. Nevertheless, the differences between the tree species were similar when results were expressed on a soil volume basis (results not shown). The stimulatory effect of birch on decomposition processes has been shown previously. Mikola (1985) found that the rate of cellulose decomposition was much faster under birch (*Betula pendula* and *Betula pubescens*) stands than under spruce (*Picea abies*) growing in originally similar soils. Also soil respiration and enzymatic activity were somewhat higher under birch. He concluded that the litter of birch is decomposed faster than that of spruce, due to its different composition, and the indirect effects of birch on soil, i.e. thermal and light conditions under birch canopy and the ground vegetation establishing on a stand, are more favourable for microbial decomposers under birch than under spruce. Bradley and Fyles (1995) found that basal respiration was 2-3 times higher in soils where birch (*Betula papyrifera*) root systems had grown compared to black spruce (*Picea mariana*) and four other tree species. They suggested the reason to be the higher amounts of root-labile C which birch may be postulated to release to the soil because of its faster growth rate compared to other tree species. Pine appears from our study to be an intermediate between spruce and birch regarding effects on soil microbes.

The stimulatory effects of birch on soil microbes depended also on soil characteristics, at the VT-site the larger microbial biomass and faster C mineralization rate were seen only in the humus layer and not in the mineral soil layers. The depthwise distribution of roots may vary in different sites, it is possible that in the mixed soil of OMT-site roots of birch have extended deeper than at the typical podzol profile of the VT-site. Usually roots of birch extend deeper than those of pine and especially spruce, the roots of which are mostly in the surface soil (Laitakari, 1927; 1934).

Microbial community structure was also influenced by the tree species, as shown by PLFAs, plate counts and CLPPs. PLFAs were grouped according to the tree species even from the two quite different sites (Fig. 2). The changes in PLFA composition due to different tree species were mainly similar in both soil layers. An exception was PLFA 10Me18:0, typical for actinomycetes (Kroppenstedt, 1985), which clearly increased in the humus layer of birch soil, but in mineral soil did not differ under different tree species. The higher pH in the birch soil and at the OMT-site (Priha and Smolander, 1999) might be one explanation for that since actinomycetes are known to have a higher pH optimum than other bacteria or fungi (Killham, 1994). PLFAs 18:1ω7 and 16:1ω7c, common in Gram-negative bacteria, for instance *Pseudomonas* species (Haack *et al.,* 1994), were also relatively more abundant in the birch soil. PLFA 16:1ω5, which is present in bacteria (Nichols *et al.,* 1986) and in arbuscular mycorrhizal fungi (Olsson *et al.,* 1995), as well as 20:4, which is found from eucaryotic organisms (Federle, 1986), were common in the birch soil from the OMT-site. The presence of 16:1ω5 could be due to the abundance of grasses in the birch plot, which can have arbuscular mycorrhizas. In mineral soil the microbial communities under birch and pine trees were not separated by the PLFA pattern.

The relative amount of PLFAs 16:1ω7t and anteiso-branched a17:0 increased in spruce soil and the amount of branched i16:0, i16:1 and 10Me16:0, which are typical for Gram-positive bacteria (O'Leary and Wilkinson, 1988), was higher in spruce and pine soils compared to birch. Branched fatty acids have previously been found to increase as a result of simulated acid rain leading to a decrease in soil pH (Pennanen *et al.,* 1998), which is in accordance with the results of this study, as spruce plots had the lowest and birch plots the highest pH (Priha and Smolander, 1999). An increased ratio of 16:1ω7t to 16:1ω7c in spruce soil could refer to stress in spruce soil since an increase in the ratio of *trans/cis* PLFAs has been suggested to indicate starvation (Guckert *et al.,* 1986) or desiccation (Kieft *et al*., 1994) in bacterial community. This is consistent with the lower microbial biomass and C mineralization rate under spruce. Nevertheless the effect was not as straightforward as that, because in the mineral soil of the VT-site spruce did not differ from pine and spruce, and C mineralization rate did not differ between pine and spruce.

The grouping of samples by CLPPs was not clear (Fig. 3). Replicate soil samples varied greatly in the rate and extent of their substrate use. Other studies have also shown that PLFAs can be more sensitive in detecting shifts in microbial community structure than Biolog profiles (Buyer and Drinkwater, 1997; Bååth *et al.,* 1998; Pennanen et al., 1998). This is probably due to the fact that PLFA analysis assesses the whole community, whereas CLPPs, using Biolog, only measures the metabolic profiles of culturable bacteria (Garland and Mills, 1991). However, some differences were seen in the CLPPs between tree species in the mineral soil samples (Fig 4). This also indicates differences in microbial community structure between the tree species, and suggests a differential availability of various C sources between stands of different tree species, which can be due to differences in the chemical composition of litter (Nykvist 1963, Johansson 1995), or root exudates (Leyval and Berthelin, 1993; Grayston *et al*., 1996). Different mycorrhizal symbionts on the roots of these trees can also significantly alter the chemical, physical and microbial composition of the rhizosphere (Rambelli, 1973; Linderman, 1988). In addition, the understorey vegetation was different under different tree species, consisting of herbs and dwarf shrubs under pine, of mosses and dwarf shrubs under spruce, and of grasses and herbs under birch. Both the exudates and the amount and quality of litter of the understorey vegetation affects microbial populations. Ericaceous species often contain high amounts of aromatic acids, polyphenols and monoterpenoids, whereas herbaceous species contain relatively low amounts of these compounds (Barford and Lajhta, 1992; Gallet and Lebreton, 1995).

CLPPs, however, measures potential utilisation and therefore care must be taken when extrapolating data to actual C source availability in the soil (Garland, 1996). Campbell *et al.* (1997) obtained a more distinctive discrimination of microbial communities of different grassland sites using the exudate sources alone than all 125 C sources, but in our study this was not the case (Figs 3, 4). Bååth *et al.* (1998) suggested that Biolog technique probably works better in environments like the rhizosphere, where a larger part of the community is active compared to bulk soil. At field sites, the situation is much more complex than in the rhizosphere.

Despite changes in microbial biomass and community structure, no tree species specific changes were observed in the FTIR spectra (Fig. 6). Microbial biomass represented approximately 2% of total soil organic C. It is plausible that the microbial pool in soil is the one responding quicker to changes than the whole organic matter pool. In addition, in heterogeneous materials, such as soil, only some of the major classes of substances and chemical compounds can be identified with FTIR spectroscopy, and only very profound changes can be seen. It was possible to predict clay content, cation-exchange capacity, carbonate content and organic matter content with near infrared spectra from arid soils in Israel (Ben-Dor and Banin, 1995). Haberhauer *et al.* (1998) compared three organic soil layers with FTIR spectroscopy and obtained similar results for all three soils. From L to H horizon they found a decrease of peak intensity at 1510 cm^{-1}, which correlated to the total C content and C:N ratio of the soil. The different C:N ratios of the soil from OMT- and VT-site could thus influence their separation form each other. It is likely that more specific groups in the organic matter could change due to tree species. Howard *et al.* (1998) studied two tree species growing in two different soils, and found that four chemical variables of the fourty-one studied were influenced significantly by tree species alone. These variables were the content of O in the humic acid, the atomic O to C ratio, the amount of vanillic acid, and the vanillic acid to protocatechuic acid ratio. Such specific changes, however, cannot be revealed with IR-spectroscopy without sample pretreatment.

In summary, soils under different tree species differed in their microbial characteristics, although the site was as important or even more important in determining the microbial and chemical characteristics of the soils. C_{mic} and C mineralization rate tended to be lowest under spruce and highest under birch, at the fertile site in all soil layers and at the less fertile site in the humus layer. Microbial community structure in both humus and mineral soil layers of the stands, determined by PLFAs, was different in soils under different tree species and suggested birch stands favoured the growth of Gram-negative bacteria such as pseudomonads. This was supported by the higher numbers of these bacteria cultured from the birch soils. At pine and spruce stands Gram-positive species appeared to be more common. The grouping of microbial communities by CLPPs was not clear, but birch soils had a tendency of separating from the others in the mineral soils. The characteristics of the organic matter of the soils, determined by FTIR spectra, did not separate the tree species.

Acknowledgements - We are grateful for Véronique Maeker, Eileen J. Reid and Ruth MacDougall for their help in the laboratory work. The Academy of Finland, and the foundations Metsämiesten säätiö and Emil Aaltosen säätiö are thanked for supporting this work financially.

REFERENCES

Anderson J. P. E. and Domsch K. H. (1978) A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10, 215-221.

Bååth E., Díaz-Raviña M., Frostegård Å. and Campbell C. (1998) Effect of metal-rich sludge amendments on the soil microbial community. Applied and Environmental Microbiology 64, 238-245.

Barford C. and Lajhta K. (1992) Nitrification and nitrate reductase activity along a secondary successional gradient. Plant and Soil 145, 1-10.

Bauhus J., Paré D. and Côté L. (1998) Effects of tree species, stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. Soil Biology and Biochemistry 30, 1077-1089.

Ben-Dor E. and Banin A. (1995) Near-infrared analysis as a rapid method to simultaneously evaluate several soil properties. Soil Science Society of America Journal 59, 364-372.

Beuker E. (1994) Long-term effects of temperature on the wood production of *Pinus sylvestris* L. and *Picea abies* (L.) Karst. in old provenance experiments. Scandinavian Journal of Forest Research 9, 34-45.

Binkley D. and Valentine D. (1991) Fifty-year biogeochemical effects of green ash, white pine and Norway spruce in a replicated experiment. Forest Ecology and Management 40, 13-25.

Bradley R. L. and Fyles J. W. (1995) Growth of paper birch (*Betula papyrifera*) seedlings increases soil available C and microbial acquisition of soil-nutrients. Soil Biology and Biochemistry 27, 1565-1571.

Buyer J. S. and Drinkwater L. E. (1997) Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. Journal of Microbiological Methods 30, 3-11.

Cajander A.K. (1949) Forest types and their significance. Acta Forestalia Fennica 56, 1-71.

Campbell C. D., Grayston S. J. and Hirst D. J. (1997) Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. Journal of Microbiological Methods 30, 33-41.

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

Frostegård Å., Bååth E. and Tunlid A. (1993) Shifts in the structure of soil microbial communities in limed forest as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25, 723-730.

Frostegård Å. and Bååth E. (1993) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59-65.

Gallet C. and Lebreton P. (1995) Evolution of phenolic patterns in plants and associated litters and humus in a mountain forest ecosystem. Soil Biology and Biochemistry 27, 157-165.

Garland J. L. (1996) Analytical approaches to the characterisation of samples of microbial communities using patterns of potential source utilisation. Soil Biology and Biochemistry 28, 213-221.

Garland J. L. and Mills A. L. (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Applied and Environmental Microbiology 57, 2351-2359.

Gerasimowicz W. V., Byler D. M. and Piotrowski E. G. (1983) Infrared spectroscopic characterization of sludges and sludge extracts: comparison of several mild extraction procedures. Soil Science 136, 237-249.

Grayston S. J. and Campbell C. D. (1996) Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (*Larix eurolepis*) and Sitka spruce (*Picea sitchensis*). Tree Physiology 16, 1031-1038.

Grayston S. J., Vaughan D. and Jones D. (1996) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5, 29-56.

Guckert J. B., Hood M. A. and White D. C. (1986) Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: Increases in the *trans/cis* ratio and proportions of cyclopropyl fatty acids. Applied and Environmental Microbiology 52, 794-801.

Haack S. K., Garchow, H., Odelson, D. A., Forney, L. J. and Klug M. J. (1994) Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. Applied and Environmental Microbiology 60, 2483-2493.

Haberhauer G., Rafferty B., Strebl F. and Gerzabek M. H. (1998) Comparison of the composition of forest soil litter derived from three different sites at various decompositional stages using FTIR spectroscopy. Geoderma 83, 331-342.

Howard P. J. A., Howard D. M. and Lowe L. E. (1998) Effects of tree species and soil physicochemical conditions on the nature of soil organic matter. Soil Biology and Biochemistry 30, 285-297.

Johansson M.-B. (1995) The chemical composition of needle and leaf litter from Scots pine, Norway spruce and white birch in Scandinavian forests. Forestry 68, 49-62.

Kieft T. L., Ringelberg D. B. and White D. C. (1994) Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Applied and Environmental Microbiology 60, 3292-3299.

Killham K. (1994) Soil Ecology. Cambridge University Press, Cambridge. 242 p.

Kroppenstedt R. M. (1985) Fatty acid and menaquinone analysis of actinomycetes and related organisms. In: Chemical Methods in Bacterial Systematics (M. Goodfellow and D. E. Minnikin Eds.), pp. 173-199. Academic Press, London.

Laitakari E. (1927) Männyn juuristo, morfologinen tutkimus. Acta Forestalia Fennica 33, 1-306.

Laitakari E. (1934) Koivun juuristo. Acta Forestalia Fennica 41, 1-216.

Leyval C. and Berthelin J. (1993) Rhizodeposition and net release of soluble organic compounds by pine and beech seedlings inoculated with rhizobacteria and ectomycorrhizal fungi. Biology and Fertility of Soils 15, 259-267.

Linderman R. G. (1988) Mycorrhizal interactions with the rhizosphere microflora: The mycorrhizosphere effect. Phytopathology 78, 366-371.

Lin-Vien D., Colthup N. B., Fateley W. G. and Grasselli J. G. (1991) The handbook of infrared and raman characteristic frequencies of organic molecules. Academic Press Limited, London. 503 p.

Liu X. and Ryan D. K. (1997) Analysis of fulvic acids using HPLC/UV coupled to FT-IR spectroscopy. Environmental Technology 18, 417-424.

Mikola P. (1985) The effect of tree species on the biological properties of forest soil. National Swedish Environmental Protection Board 3017, 1-29.

Nichols P. D., Stulp B. K., Jones J. G. and White D. C. (1986) Comparison of fatty acid content and DNA homology of the filamentous gliding bacteria *Vitreoscilla*, *Flexibacter*, and *Filibacter*. Archives of Microbiology 146, 1-6.

Nykvist N. (1963) Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter. Studia Forestalia Suecica 3, 1-31.

O'Leary W.M. and Wilkinson S.G. (1988) Gram-positive bacteria. In: Microbial Lipids, vol 1. (Ratledge, C. and Wilkinson, S.G., Eds.) pp. 117-202. Academic Press Ltd., London.

Olsson P. -A., Bååth E., Jakobsen I. and Söderström B. (1995) The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycological Research 99, 623-629.

Pennanen T., Fritze H., Vanhala P., Kiikkilä O., Neuvonen S. and Bååth E. (1998) Structure of a microbial community in soil after prolonged addition of low levels of simulated acid rain. Applied and Environmental Microbiology 64, 2173-2180.

Priha O. and Smolander A. (1997) Microbial biomass and activity in soil and litter under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at originally similar field afforestation sites. Biology and Fertility of Soils 24, 45-51.

Priha O. and Smolander A. (1999) Nitrogen transformations in soil under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. Soil Biology and Biochemistry 31: 965-977 (in press).

Rambelli A. (1973) The rhizosphere of mycorrhizae. In: Ectomycorrhizae: Their Ecology and Physiology (G. C. Marks and T. T. Kozlowski, Eds), pp. 299-243. Academic Press, New York.

Senesi N., Miano T. M. and Martin J. P. (1987) Elemental, functional infrared and free radical characterization of humic-acid type fungal polymers (melanins). Biology and Fertility of Soils 5, 120-125.

Stevenson F. J. and Goh K. M. (1971) Infrared spectra of humic acids and related substances. Geochimica et Cosmochimica Acta 35, 471-483.

Sparling G. P., Feltham C. W., Reynolds J., West A. W. and Singleton P. (1990) Estimation of soil microbial C by a fumigation-extraction method: use on soils of high organic matter content, and a reassessment of the k_{ec}-factor. Soil Biology and Biochemistry 22, 301-307.

Turner D. P., Sollins P., Leuking M. and Rudd N. (1993) Availability and uptake of inorganic nirogen in a mixed old-growth coniferous forest. Plant and Soil 148, 163-174.

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

West A. W., Sparling G. P. (1986) Modifications to the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents. Journal of Microbiological Methods 5, 177-189.

Paper IV

Priha O., Lehto T. & Smolander A. (1998) Mycorrhizas and C and N transformations in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings. Plant and Soil 206: 191-204 (in press). © 1998 Kluwer Academic Publishers.
Reprinted with kind permission from Kluwer Academic Publishers.

IV

Statement of the contribution of the individual authors in paper entitled

Mycorrhizas and C and N transformations in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings

by

Outi Priha, Tarja Lehto* and Aino Smolander

Finnish Forest Reasearch Institute, P.O.Box 18, FIN-01301 Vantaa, Finland
*University of Joensuu, Faculty of Forestry, P.O.Box 111, FIN-80101 Joensuu, Finland

Which was accepted for publication in Plant and Soil October 22, 1998.

Contributions of the authors listed are:

Outi Priha: Corresponding author. Planning and establishing the pot experiment. Performing experimental work, except for mycorrhizal work. Responsible for writing and interpretation of the results.
Tarja Lehto: Performing and interpreting the mycorrhizal determinations.
Aino Smolander: Supervisor of the Ph.D. studies of Outi Priha.

All authors have given their comments of the paper before submission.

Outi Priha

Tarja Lehto

Aino Smolander

Plant and Soil **00**: 1–14, 1998.

Mycorrhizas and C and N transformations in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings

O. Priha[1], T. Lehto[2] & A. Smolander[1] *1

[1]*Finnish Forest Research Institute, P.O. Box 18, FIN-01301 Vantaa, Finland**; [2]*University of Joensuu, Faculty of Forestry, P.O. Box 111, ~~FLN~~ FIN-80101 Joensuu, Finland*

Received ???. Accepted in revised form ??? 22 October, 1998
24 October, 1997

Key words: C transformations, mycorrhiza, N transformations, rhizosphere, roots, tree species

Abstract

Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.) and silver birch (*Betula pendula* L.) seedlings were grown in a greenhouse for four months in three different soils. The soils were from a field afforestation site on former agricultural land: soil from a pine site, soil from a spruce site and soil from a birch site. Pots without seedlings were included. The aim was to discover, independent of the effects of the different quality of aboveground litter and microclimate under the tree species, whether the roots change the microbial activities and chemical characteristics of the soil, whether the changes are dependent on the tree species, and whether the changes vary in different soils.

Pine, spruce and birch had, on average, five, one and six meters of roots, respectively. Birch had by far the highest number of short root tips, on average 11 450 per seedling, compared to 1900 and 450 in pine and spruce seedlings, respectively. The majority of the short roots of pine and spruce were brown sheathed mycorrhizas, and those of birch were mycorrhizas in an early stage of development.

The seedlings caused no major changes in either the soil pH or the concentrations of nutrients in the soils, but did affect the microbial characteristics of the soils. The effect of the tree species did not differ in different soils. Microbial biomass C and N, C mineralization rate and the concentration of ergosterol were all higher under birch and pine than under spruce and in plantless soils. Nitrate concentrations were lowest under pine and birch, but rates of net N mineralization, nitrification and denitrification did not differ under different seedlings.

The stimulative effect of pine and especially birch on soil microbes was possibly due to them having more roots and releasing more root exudates to soil. There were, however, indications that not only the length/mass of roots determined the changes in microbial activities, but also differences in root activities per unit of root or in the quality of root exudates.

Introduction

Plant cover and especially the dominant tree species have fundamental effects on biological and chemical soil properties. Some tree species, like Norway spruce, may cause the cycling of nutrients to slow down (Mikola, 1985; Ranger and Nys, 1994), whereas others, like birch species, may improve the soil fertility (Mikola, 1985; Bradley and Fyles, 1995; Miles and Young, 1980). Similar effects were also found in soil under Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.) and silver birch (*Betula pendula* L.) in field sites where these trees had been planted in originally similar forest soil approximately 60 years ago (unpublished results). The soil pH, base saturation, microbial biomass C and N, and C mineralization were all highest under birch and lowest under spruce. These same tree species had not, however, had a clear differential effect on soils of field afforestation sites planted 23–24 years ago (Priha and Smolander, 1997). Nevertheless, the ground vegetation was differentiated under

* FAX No: + 3589 857 2575. E-mail: Outi.Priha@metla.fi

different tree species, and microbial characteristics of their litters varied.

Trees affect the soil by their litter, root activity and associated microclimate, and in field studies it is not possible to separate these effects from each other. The positive effect of birch on the soil and the negative effect of spruce have often been explained to be due to the quality of their litters. Birch leaf litter has a larger content of water-soluble substances and simple carbohydrates and also a somewhat higher pH and concentration of base compounds than coniferous needle litter (Berg and Wessén, 1984; Nykvist, 1963). However, it has also been shown that the beneficial effect of birch may arise from the activities of its roots, especially the large amount of labile C released to the soil (Bradley and Fyles, 1995). The effects of roots on soil include uptake of nutrients and water, exudation and other secretions, turnover, and movement of soil caused by root penetration. In pot experiments the effect of roots can be studied separately from the other effects of the trees.

The aim of this study was to determine whether roots of Scots pine, Norway spruce and silver birch seedlings alter the microbial activities and chemical characteristics of soils, whether the changes are dependent on the tree species, and whether the changes vary in different soils. The aim was also to characterise the roots and mycorrhizas of pine, spruce and birch seedlings in different soils.

Materials and methods

Soils

The soils were taken from a 25-year-old field afforestation experiment on former agricultural land, established by Leikola (1977). The original soil texture was silty fine sand. The experiment was situated in Karttula (62°52′ N, 27°10′ E, 98 m above sea level), and contained three blocks (randomized block design) divided into one plot each (30×64 m) of Scots pine, Norway spruce and silver birch. This experiment has been described in detail in Priha and Smolander (1997). Soil was collected in September 1994 from the uppermost 10 cm from 40 randomly-selected places in each plot within block 2 and combined plotwise. In the laboratory green plant material was removed and soils were sieved (mesh size 5.7 mm) and stored frozen (−18 °C) during the winter.

Pot experiment

The pot experiment was started in May 1995. A mixture of soil (150 mL) and gravel (for maintaining the aeration of soil; grain size 3–5 mm, 150 mL) was put into 350 mL pots with a layer of gravel and holes in the bottom. Seeds of Scots pine, Norway spruce and silver birch, of Southern Finnish provenances, were surface sterilized with 30% H_2O_2 for 15 min and rinsed with sterile water five times. Four to five seeds were sown into each pot, but after the seeds had germinated, seedlings were thinned to one seedling per pot. Altogether there were 12 treatments: pine, spruce and birch growing in pine, spruce and birch soil, and all three soils without seedlings.

The experiment had a randomized block design with 30 pots per treatment divided evenly into five blocks. The blocks were based on a gradient of light. In the greenhouse, the temperature was 22 °C during the day (16 h) and 18 °C during the night (8 h). Na-lamps (Elektro-valo) were used during the daytime when the light intensity decreased below 200 W m^{-2}. Seedlings were watered to saturation approximately every other day.

Harvest

Four months after the germination of the seeds (September 1995), five pots of each treatment, i.e., one randomly selected from each block, were harvested for soil analyses. In the laboratory seedlings were removed from the pots, and the roots were shaken and brushed gently to remove the adhering soil. All of the soil from each pot was analysed. An additional five pots of each treatment, one from each block, were harvested for counting and classifying the root tips. The rest of the pots were saved to be analysed in later studies.

Analyses of the seedlings

The shoots were dried at 40 °C for 48 h and weighed. The needles/leaves were separated and ground for analysis of total N and P. Ground foliage material from the five seedlings harvested for soil analyses, and from the five seedlings used for the determination of short root tips, were each combined to give two samples per treatment. Total N was determined with the Kjehdahl method, modified by Kubin and Siira (1980), and measured spectrophotometrically. For determination of total P, the plant material was ignited and the ash was dissolved in HCl. Total P of the extractant was determined spectrophotometrically with the vanado-molybdate method (Halonen et al. 1983).

Roots from the soil analyses pots were frozen in water and subsequently their length was measured by scanning them with Mac/WinRHIZO V3.0.2 program (1995). After scanning, the roots were dried at 65 °C for 48 h and weighed.

Counting and classifying the root tips

Roots were washed free of soil with water (soil was discarded) and long root tips, short root tips and recently emerged root tips were counted using a binocular microscope (Wilcox, 1964). Short root tips were further classified into those without mycorrhizal infection, developing mycorrhizas (early stage of colonization, usually Hartig net developed but mantle not yet covering the whole short root, as seen after bleaching; de la Rosa et al., 1998), brown sheathed mycorrhizas, *Cenococcum geophilum* (Mikola, 1948), dichotomous, and other mycorrhizas. Every short root of pine and spruce root systems was examined. Subsamples of birch root systems were counted, and the number in the whole root system was estimated using the dry masses of the subsample and the whole root system. Three major laterals comprised the birch subsamples, one from the upper part, one from the middle part and one from the lower part of the major root. If these three laterals did not have at least 300 long root tips, then another lateral was selected randomly. After classification the roots were dried at 65 °C for 48 h and weighed.

Chemical analyses of the soils

The dry weight (d.w.) of soil was determined by drying the samples at 105 °C for 24 h. Soil organic matter content was measured as loss on ignition from the dried samples at 550 °C for 4 h. Water-holding capacity (WHC) was measured by soaking the soil samples in water for 2 h and then draining for 2 h. Soil pH was measured in soil 0.01 *M* $CaCl_2$ suspensions (3:5 v/v).

For determination of total N, the soils were dried at 40 °C for 48 h, and the gravel was sieved away. Total N was determined by the Kjeldahl method (Halonen et al., 1983). For determination of soluble P and exchangeable K, Ca and Mg, 10 mL fresh soil samples were extracted with 100 mL 0.5 *M* CH_3COONH_4 (pH 4.65) and nutrients subsequently measured by an inductively-coupled plasma emission spectrometer (ARL 3580).

Microbial analyses of the soils

For microbial measurements, 10 g d.w. soil samples with the soil moisture content adjusted to 60% of the WHC were used, unless otherwise stated. Gravel was not separated from the soil for any of the measurements, but afterwards the mass of gravel from each bottle was determined and subtracted from the mass of soil for calculation of the results.

The measurements of microbial biomass C and N with fumigation-extraction (FE) and microbial biomass C with substrate-induced respiration (SIR) methods were based on the approaches of Vance et al. (1987) (FE-C), Brookes et al. (1985) (FE-N) and Anderson and Domsch (1978) and West and Sparling (1986) (SIR). These results were otherwise shown without the use of conversion factors, but to determine how large a proportion microbial biomass is of total soil organic C and total N, the flushes of C and N from fumigation were converted to microbial biomass C and N with a k_{ec} factor 0.35 (Sparling et al., 1990) and a k_n factor 0.54 (Brookes et al., 1985). C mineralization was evaluated as CO_2-C production in a 48 h incubation at 20 °C. Net ammonification and nitrification were studied in a 40-day aerobic incubation at 20 °C. Nitrogenase (N_2 fixing) activity was assessed with the acetylene reduction method. All of the above methods are described in detail in Priha and Smolander (1997).

Denitrification potential was measured as N_2O production. The soil moisture content was adjusted to 100% of the WHC and the samples were incubated at 20 °C in 125 mL glass bottles under 10 kPa partial pressure of acetylene. The N_2O evolved was measured after 48 and 72 h incubation with a gas chromatograph (Hewlett Packard 6890 Series), equipped with an electron capture detector and a Megapore GS-Q column (J&W Scientific), 30 m in length, using He (10 mL min^{-1}) as carrier gas and $ArCH_4$ (95:5) as make-up gas. The temperatures of the detector, injector and oven were 300, 100 and 30 °C, respectively. The results are given as production rate of N_2O-N between 48 and 72 h. The solubility of N_2O in water was taken into account in the calculations (Moraghan and Buresh, 1977).

Ergosterol content of the soils, for determination of the fungal biomass, was measured using the method of Nylund and Wallander (1992), as modified by Olsson et al. (1996). From each soil, a 2 g d.w. sample with 7-dehydrocholesterol (50 μL of a 0.91 μg μL^{-1} solution in methanol) added as an internal standard, was extracted with 2 mL methanol by vortexing for

10 s, sonicating for 2 ~~mm~~ min, and vortexing for 10 s. The extract was centrifuged for 10 min at 1000 *g*, the supernatant removed, and the pellet washed twice with 2 mL methanol. The supernatants were combined and 1.1 mL of 4% KOH in ethanol (freshly made) was added, and the mixture was saponified for 30 min in 80 °C waterbath. After cooling, distilled water and hexane (2 mL each) were added, and the samples turned up and down 20 times. The hexane phase was separated from the water phase, and the water phase washed once with 2 mL hexane. The combined hexane extract was dried under N_2 gas. The extracted sterol material was dissolved in 1 mL acetonitrile and analysed by high-pressure-liquid-chromatography (HPLC) (Merck-Hitachi L-6200 Intelligent pump and L-4250 UV-VIS detector). The HPLC was equipped with a LiChrospher 100 RP-18 column (Hewlett-Packard) at 25 °C, using hexane-isopropanol-acetonitrile (5:5:90) with a flow-rate of 1 mL min^{-1} as a carrier. Ergosterol was detected at 282 nm. All chemical solvents were of HPLC grade.

Statistical analyses

The aim of this study was to determine whether roots of pine, spruce and birch seedlings altered the characteristics of soils (tree species effect) and whether these changes varied in different soils (tree species×soil interaction). We also wanted to see whether different soils affected the characteristics of the seedlings (soil effect). Means of the measured characteristics of the samples were compared using analysis of variance with tree species, soil and block as main effects and testing also the interaction between tree species and soil (Ranta et al., 1989). Results were log transformed when necessary for fulfilling the assumptions of analysis of variance. An angular transformation (arcsin $\sqrt{x}$) was performed on the percentages of different short root tips. Significant ($p \leq 0.05$) differences of the means by tree species or by soil were separated by Tukey's test (HSD, honestly significant difference), using a signifigance level of $p \leq 0.05$ (Ranta et al., 1989). When the interaction between tree species and soil was significant, means of all treatments were compared pairwise by Tukey's test. Correlations between microbial and root variables were tested with Pearson's correlation (Ranta et al., 1989).

Results

Seedlings

Pine and birch seedlings had, on average, a dry mass of 60 and 63 mg, respectively. The average dry mass of spruce seedlings was only 10 mg. The concentration of N was, on average, 24.7, 31.7 and 15.3 mg g^{-1} d.w. in pine needles, spruce needles and birch leaves, respectively, and the concentration of P was, on average, 2.7, 4.6 and 3.3 mg g^{-1} d.w. in pine needles, spruce needles and birch leaves, respectively.

Root weight ratio (root weight divided by total weight of the seedling) was, on average, 0.34, and was highest in birch seedlings (Table 1). The numbers of short root tips were highest in birch seedlings and lowest in spruce seedlings. Root length did not vary significantly between pine and birch, but was lowest in spruce. Soil did not affect these root variables.

The percentages of all types of short root tips varied significantly in different tree species (Table 1). The majority of the short roots of pine and spruce were brown sheathed mycorrhizas, and the majority of those of birch were developing mycorrhizas. Birch seedlings were the only ones which had non-mycorrhizal short root tips. There were very few *Cenococcum geophilum* mycorrhizas in birch and pine and none in spruce. In the brown sheathed mycorrhiza, the interaction between tree species and soil was significant, but birch had the lowest percentage of brown sheathed mycorrhizas in all soils and spruce the highest. The dichotomous short root tips, typical only for pine, were most abundant in pine soil, and least abundant in birch soil.

Physical and chemical characteristics of the soils

No major changes due to tree species were found in physical and chemical characteristics of the soils (Table 2).

Microbial biomass C and N, substrate-induced and basal respiration

Tree species had a significant effect on the flushes of C and N from fumigation; both were higher under birch than in soils under spruce and without seedlings (Figure 1, Table 3). Microbial biomass C was, on average, 1.3% of soil organic C and microbial biomass N 2.0% of total soil N. Microbial biomass under birch contained a significantly larger proportion of soil organic C ($p = 0.01$) and total soil N ($p < 0.00$) than in soil under spruce and without seedlings. The soil microbial

Table 1. Some characteristics of the seedlings and different types of short root tips as percentages. Values are means of five seedlings, st/andard errors of the means in parentheses. Brown sheathed mycorrhiza; values with the same letter are not significantly different from each other ($p \leq 0.05$).

Soil	Tree species	df	Root weight ratio (root weight / total weight)	Root length, cm	Number of short root tips	Non-mycorrhizal	Developing mycorrhiza	Brown sheathed mycorrhiza	*Cenococcum geophilum*	Dichotomous	Others
Pine soil	Pine		0.30 (0.01)	410 (24)	1643 (177)	0 (0)	2 (1)	60 (8) d	0 (0)	33 (6)	5 (3)
	Spruce		0.29 (0.01)	106 (27)	688 (241)	0 (0)	2 (1)	98 (1) a	0 (0)	0(0)	0 (0)
	Birch		0.39 (0.02)	612 (91)	9104 (1132)	2 (0)	76 (2)	18 (1) e	3 (2)	0 (0)	1 (0)
Spruce soil	Pine		0.32 (0.02)	522 (49)	1918 (346)	0 (0)	1 (1)	77 (4) cd	1 (0)	20 (9)	1 (0)
	Spruce		0.29 (0.02)	86 (19)	370 (104)	0 (0)	4 (2)	96 (2) a	0 (0)	0(0)	0 (0)
	Birch		0.43 (0.02)	690 (76)	11422 (2677)	3 (1)	66 (4)	24 (82) e	3 (1)	0 (0)	4 (1)
Birch soil	Pine		0.32 (0.01)	495 (50)	2152 (325)	0 (0)	1 (0)	86 (1) bc	0 (0)	12 (2)	1 (1)
	Spruce		0.34 (0.02)	103 (27)	286 (58)	0 (0)	6 (2)	94 (3) ab	0 (0)	0 (0)	0 (0)
	Birch		0.38 (0.03)	659 (108)	13807 (1882)	3 (1)	79 (3)	15 (3) e	1 (0)	0(0)	2 (1)
Anova	Source of variation	df	p-value								
	Tree species	2 ~~0.00~~	0.00	0.00	0.00	0.00	0.00	0.00	0.00	~~0.00~~	0.00
	Soil	2	0.25	0.89	0.63	0.92	0.13	0.14	0.14	0.02	0.59
	Tree species × soil	4	0.15	0.81	0.39	0.99	0.19	0.00	0.34		0.03

Table 2. Some chemical characteristics of the soils at the end of the experiment. Values are means of 15 pots (5 with pine soil, 5 with spruce soil, and 5 with birch soil), standard errors of the means in parentheses

Tree species		pH ($CaCl_2$)	Organic matter, % of d.w.	Organic matter, % of d.w.	Total N, $mg\ g^{-1}$ o.m.	Exchangeable nutrients, $mg\ g^{-1}$ o.m.			
		(with gravel)	(without gravel)		P	K	Ca	Mg	
Pine		4.4 (0.0)	3.6 (0.1)	11.3 (0.3)	26.4 (0.4)	0.062 (0.003)	0.58 (0.03)	12.3 (0.7)	1.48 (0.13)
Spruce		4.4 (0.0)	3.5 (0.1)	11.1 (0.3)	26.5 (0.7)	0.064 (0.003)	0.57 (0.05)	12.3 (0.7)	1.37 (0.11)
Birch		4.4 (0.0)	3.8 (0.0)	11.3 (0.2)	26.7 (0.7)	0.060 (0.004)	0.60 (0.02)	12.1 (0.6)	1.43 (0.08)
No seedling		4.3 (0.0)	3.6 (0.1)	11.2 (0.3)	26.4 (0.5)	0.066 (0.002)	0.58 (0.03)	12.1 (0.6)	1.35 (0.09)
Anova									
Source of variation	df				p-value				
Tree species	3	0.00	0.64	0.25	0.64	0.07	0.01	0.37	0.01
Soil	2	0.29	0.47	0.00	0.00	0.00	0.00	0.00	0.00
Tree species × soil	6	0.10	0.80	0.40	0.64	0.67	0.00	0.62	0.18

d.w. = dry weight; o.m. = organic matter.

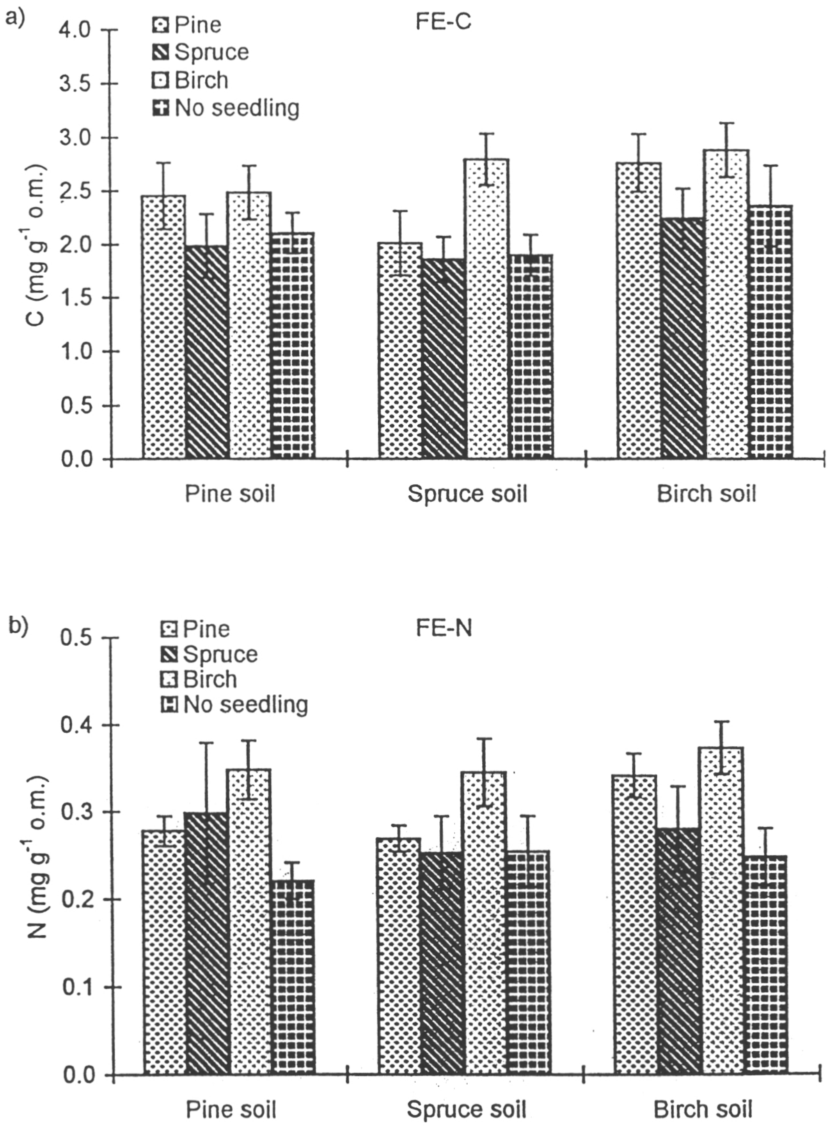


Figure 1. The flush of (a) extractable C and (b) extractable N from fumigation of the soils. Values are means of five pots, bars show standard errors of the means.

Table 3. Probabilities from analysis of variance of microbial variables

Source of variation	df	*p*-value				
		FE-C (Figure 1a)	FE-N (Figure 1b)	Basal resp. (Figure 2a)	SIR (Figure 2b)	Ergosterol (Figure 3)
Tree species	3	0.01	0.00	0.00	0.00	0.00
Soil	2	0.08	0.54	0.30	0.00	0.00
Tree species × soil	6	0.87	0.97	0.40	0.84	0.05

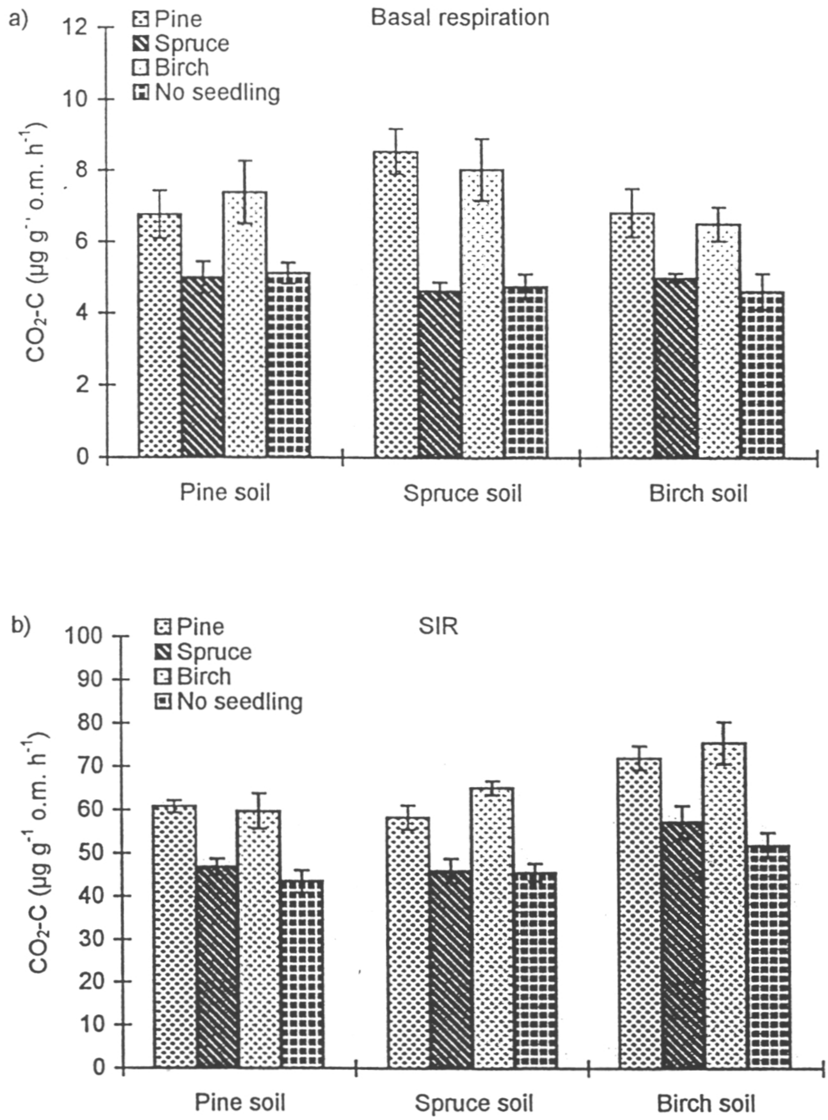


Figure 2. CO_2-C evolved from (a) basal respiration and (b) substrate-induced respiration of the soils. Values are means of five pots, bars show standard errors of the means.

C:N ratio varied between 11–15, and did not differ significantly under different tree species. Both basal respiration and substrate-induced respiration were significantly higher under pine and birch than in soils under spruce and without seedlings (Figure 2, Table 3). The above effects of the tree species did not vary in different soils.

Ammonification, nitrification, denitrification and nitrogenase activity

Neither the ammonium concentration or net ammonification rate, nor denitrification potential differed under different seedlings, but this may have been due to the large variability of the data (Table 4). Tree species had a significant effect on nitrate concentrations, which

Table 4. NH_4^+-N and $(NO_2^- + NO_3^-)$-N concentrations, net ammonification, net nitrification, and denitrification potential of the soils. Values are means of five pots, standard errors of the means in parentheses

Soil	Tree species	df	NH_4^+-N, μg g^{-1} o.m.	$(NO_2^- + NO_3^-)$-N, μg g^{-1} o.m.	NH_4^+-N production, μg g^{-1} o.m. 40 d^{-1}	$(NO_2^- + NO_3^-)$-N production, μg g^{-1} o.m. 40 d^{-1}	N_2O-N production, ng g^{-1} o.m. h^{-1}
Pine soil	Pine		21 (16)	35 (3)	−3 (16)	391 (45)	1597 (124)
	Spruce		12 (8)	78 (5)	−11 (8)	276 (46)	1204 (53)
	Birch		15 (11)	28 (25)	−8 (14)	252 (53)	839 (258)
	No seedling		16 (8)	120 (17)	−16 (8)	335 (20)	1187 (168)
Spruce soil	Pine		25 (8)	5 (3)	22 (26)	257 (40)	444 (171)
	Spruce		15 (10)	58 (7)	0 (20)	213 (14)	1146 (152)
	Birch		17 (10)	6 (4)	35 (22)	213 (81)	555 (216)
	No seedling		18 (14)	80 (9)	−4 (22)	238 (5)	867 (146)
Birch soil	Pine		16 (11)	21 (6)	23 (15)	412 (39)	1427 (259)
	Spruce		14 (10)	93 (15)	3 (19)	359 (14)	1309 (131)
	Birch		13 (7)	15 (8)	1 (15)	309 (31)	1033 (364)
	No seedling		26 (14)	143 (11)	−18 (18)	385 (21)	962 (50)
Anova	Source of variation	df	*p*-value				
	Tree species	3	0.48	0.00	0.08	0.02	0.06
	Soil	2	0.50	0.00	0.29	0.00	0.00
	Tree species × soil	6	0.77	0.25	0.69	0.90	0.10

o.m. = organic matter.

were lowest under pine and birch and highest in plantless soil. Net nitrification rate was higher under pine than under birch. Acetylene reduction (nitrogenase) activity was not detected from any of the soils (results not shown). None of these tree species effects was different in different soils.

Ergosterol concentration of soils

For ergosterol concentration, the interaction between tree species and soil was significant (Figure 3, Table 3). In all soils, however, the concentration of ergosterol tended to be higher under birch and pine than under spruce and in plantless soils. Ergosterol concentration of the soils correlated positively ($r = 0.66$, $p = 0.00$) with the number of mycorrhizal short root tips.

The relationship between microbiological variables and root variables

To evaluate the meaning of root quantity on the activity of microbes, the values for FE-C, FE-N, basal respiration and SIR in plantless pots were subtracted from the values in pots with plants (from the same block) and the differences were plotted against root length. The differences obtained from FE-C and FE-N did not correlate significantly with the root variables, but the ones from basal respiration and SIR both correlated positively with root length when all tree species were included (Figure 4) (basal respiration: $r = 0.68$, $p = 0.00$ and SIR: $r = 0.60$, $p = 0.00$). The correlations were similar when the differences were plotted against root dry weight or number of root tips. Within one tree species the differences and the root variables correlated variably: the differences obtained from basal respiration correlated positively within pine and spruce, but not within birch. The differences obtained from SIR correlated positively with root length only within birch, whereas spruce showed a negative correlation.

When these subtractions were divided by root length, it was shown that pine and birch roots caused approximately the same increase for the flushes of C and N from fumigation, basal respiration and SIR per unit of root (Table 5). Spruce decreased the flushes of

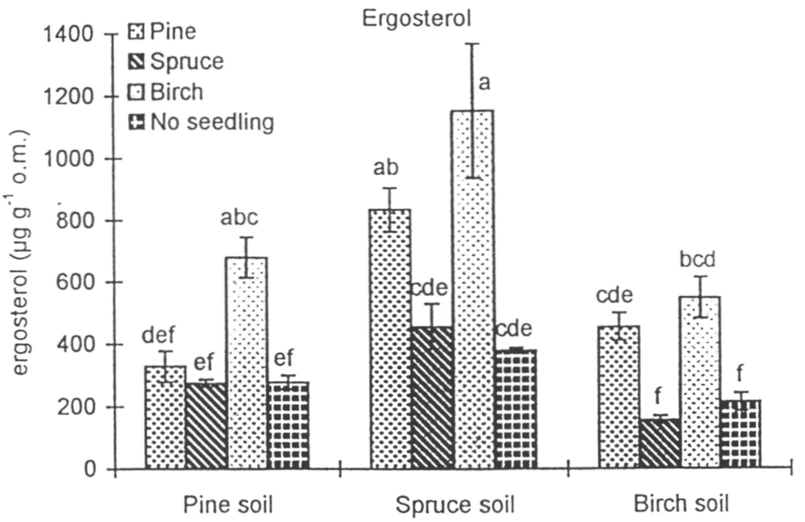


Figure 3. Ergosterol concentration of the soils. Values are means of five pots, bars show standard errors of the means. Values with the same letter are not significantly ($p \leq 0.05$) different from each other.

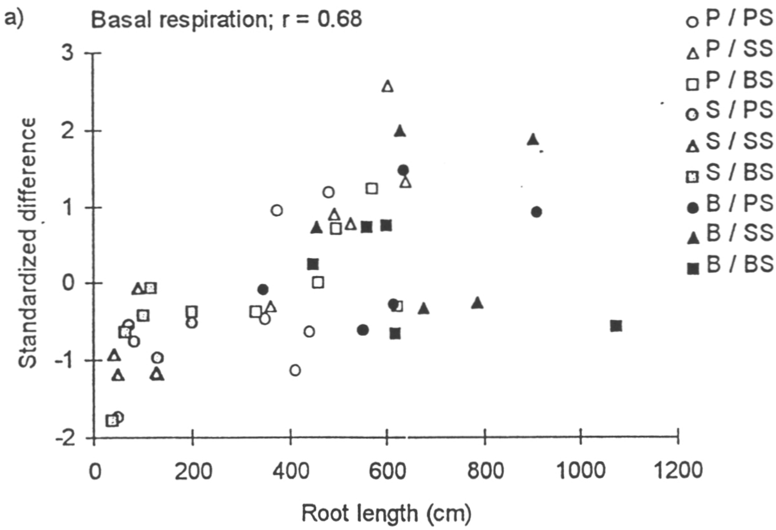
C and N from fumigation and basal respiration, but increased SIR more per unit of root than did pine and birch. These differences in the effects of tree species were statistically significant for the flush of C from fumigation and basal respiration, but not for the flush of N from fumigation and SIR.

Discussion

Differences in the chemical and microbial properties of soil have previously been observed in soils under different tree species (e.g. Bradley and Fyles, 1995; Mikola, 1985). In this pot study we wanted to determine whether the differences under Scots pine, Norway spruce and silver birch can partly be a result of their different root activities, rather than being only a result of differences in the chemical composition and decomposition rate of their litter and in the different microclimates the tree species create. At the time of harvest there were negligible amounts of dead roots in all pots. Therefore, any differences in the input of organic matter from dead fine roots probably did not affect the results.

The only influence of soil observed on mycorrhizas was the high proportion of the dichotomous branching in pine seedlings grown in pine soil, as opposed to pine growing in spruce or birch soil (Table 1). Soil originating from a pine stand would be expected to include inoculum of compatible mycorrhizal fungi for the pine seedlings, and it could be speculated that the increase in dichotomous branching is a reflection of this increased compatibility. Nevertheless, the total numbers of root tips or the developmental stage of mycorrhizas were not affected by the soil. If the mycorrhizal fungi originating from the pine soil had been more successful, trends of improved growth or nutrient uptake for the pine in pine soil treatments would have been expected. The lack of such trends indicates that the efficacy of mycorrhizas in nutrient uptake was not altered by the relatively high-nutrient soils in this experiment.

The effects of the pine, spruce and birch seedlings on soil chemical characteristics and microbial activities did not differ in different soils, but had the same trends in all soils. Seedlings had not caused any major changes either in the soil pH or in the concentrations of nutrients in the soils during one growing season (4 months) (Table 2). Seedlings had, however, affected the microbial characteristics of the soils, and the effect varied between the three tree species. Microbial biomass C and N and C mineralization rate were all higher under birch and pine than under spruce and in plantless soils (Figures 1 and 2). Trees allocate up to 40–70% of their photosynthetically assimilated C belowground, and of this amount from 2 to 10% is lost as root exudates (see review by Grayston et al., 1996). This usually causes increased microbial biomass and numbers in the rhizosphere. Pine and birch root sys-



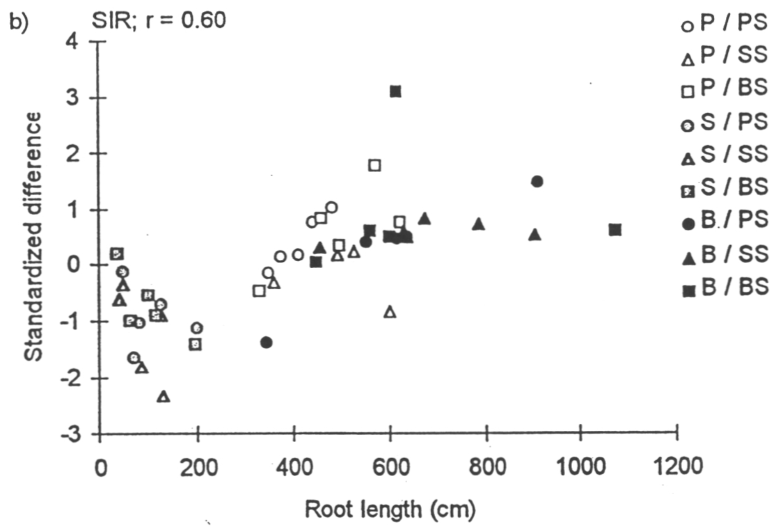


Figure 4. (a) Basal respiration and (b) substrate-induced respiration (SIR) of the soils: values from pots without seedlings subtracted from the ones with seedlings and plotted against root length. Symbols: P = pine, S = spruce, B = birch, PS = pine soil, SS = spruce soil, BB = birch soil; r = Pearson correlation coefficient.

tems were about five-fold longer than spruce root systems, and also had more root tips (Table 1), which are the locations for exudation. Thus their stimulating effect could be explained by quantitative differences; more roots and root tips supplying more root exudates. The correlations between root length, and basal respiration and SIR (Figure 4) support this hypothesis. On the other hand, the situation might not be as simple as that, as not all correlations between SIR and basal respiration and root length were positive within one tree species. In addition to the quantity of root exudates, their quality differs between different tree species (Smith, 1976). The root exudates of birch and pine could be more suitable substrates for microbial

Table 5. The flushes of C and N from fumigation (FE-C and FE-N), basal respiration and substrate-induced respiration (SIR): values from pots without seedlings subtracted from the ones with seedlings and divided by root length. Values are means of 15 pots, standard errors of the means in parentheses

Tree species		FE-C $\mu g\ g^{-1}$ o.m. cm^{-1}	FE-N $\mu g\ g^{-1}$ o.m. cm^{-1}	Basal resp. CO_2-C, $\mu g\ g^{-1}$ o.m. cm^{-1}	SIR CO_2-C, $\mu g\ g^{-1}$ o.m. cm^{-1}
Pine		4.5 (3.3)	0.7 (0.3)	0.042 (0.008)	0.27 (0.04)
Spruce		−49.7 (18.7)	−2.1 (2.9)	−0.044 (0.046)	0.63 (0.37)
Birch		7.1 (2.6)	1.6 (0.3)	0.043 (0.010)	0.39 (0.06)
Anova					
Source of variation	df		p-value		
Tree species	2	0.00	0.30	0.02	0.47
Soil	2	0.93	0.33	0.93	0.64
Tree species × soil	4	1.00	0.38	0.90	0.74

o.m. = organic matter.

growth and mineralisation than those of spruce. This hypothesis is supported by the increase in microbial biomass C and N and basal respiration per unit of root by pine and birch, and decrease by spruce (Table 5). Spruce roots did, however, increase substrate-induced respiration per unit of root, even more than pine and birch roots did, indicating a larger population of active microbes per unit of root.

Apart from birch having a larger root system, the developmental stage of birch mycorrhizas may have played a role in the larger amount and activity of soil microbes under the birch seedlings. Most of the birch mycorrhizas did not have a fully developed mantle, and therefore their fungal partners may have been less efficient in capturing root exudates. This would allow better access to exudates by other soil microbes.

Root exudates have a high C:N ratio, and even though soil microbes are generally C-limited, in the rhizosphere other nutrients, especially N, may become limiting. Roots often stimulate microbial growth by providing substrates, but roots and microbes can also compete with each other for nutrients and water. Parmelee et al. (1993) found that in organic soil, microbial growth rate and microbial biomass C and N decreased when root density of pine increased, but in mineral soil microbes were stimulated by pine roots. They suggested that in organic soil there was no C limitation, but roots limited microbial activity by reducing soil moisture and/or N availability. In contrast, in nutrient-poor mineral soil roots provided the main input of substrate, and this was more significant than the adverse effects of roots. In our study, microbial C and N were, on average, 1.3 and 2.0% of soil organic C and total soil N, respectively. Both proportions were higher in soil under pine and birch than under spruce and in plantless soils. Based on a simple division of C and N availability to the microflora by Joergensen et al. (1995), the soils under pine and birch would fall into high C availability (microbial C : soil organic C > 12 mg g^{-1}) and the others into low C availability, but all soils would have a low N availability (microbial N: total soil N < 28 mg g^{-1}). Furthermore, the microbial C:N ratio was quite high in all soils, from 11 to 15, which could also be an indication of low N availability and thus N deficiency of microbes. Most organisms contain a fairly constant proportion of C, but the N content of microbes varies widely, falling sharply if they are grown in a medium deficient in N (reviewed by Jenkinson, 1988).

Although the total amount of N in birch seedlings was high, the concentration of N in the leaves of the birch seedlings was very low (Saarsalmi et al., 1992), and N probably limited the growth of birch. The N concentration in pine and spruce needles, however, indicated a good nutritional status of the seedlings (Jukka, 1988; Rikala and Huurinainen, 1990). Thus, under birch, where C is less limiting for microbes, it seems that the competition for N between microbes and the plant is most intensive.

There was less nitrate under pine and birch than under spruce and in plantless soils, but net nitrification rates were on the same level in all soils (Table

4). Loss of nitrate via denitrification was probably not the reason, because denitrification potential was not higher under pine and birch when compared with spruce (Table 4). It may be that pine and birch had been using the produced nitrate. Although both non-mycorrhizal and mycorrhizal conifer roots generally prefer ammonium over nitrate as a source of inorganic N (Flaig and Mohr, 1992; Marschner et al., 1991; McFee and Stone, 1968), Norton and Firestone (1996) showed that mycorrhizal pine (*Pinus ponderosa*) roots were more successful competitors with microbes for limited inorganic N when the N source was nitrate vs ammonium. Either the N limitation in the rhizospheres of pine and birch had caused the use of nitrate or there are differences in the N uptake preferences of pine, spruce and birch.

The ergosterol assay has been criticised because the amount of ergosterol varies between fungal species and also within species in cells of different age (Bermingham et al., 1995). It was, however, used in this study, as it is one of the few methods of determining fungal biomass. In this experiment, the ergosterol analysis included both saprophytic soil fungi and part of the mycorrhizal mycelium. The concentration of ergosterol in soil correlated positively with numbers of mycorrhizal short root tips (Table 1, Figure 3), which is an indirect indication that mycorrhizal mycelium comprised a large proportion of soil fungi in the pots.

In conclusion, soil microbial biomass and activities were stimulated by roots of pine and birch in this study, but not by spruce roots. Pine and especially birch had more roots and thus possibly also supplied more substrates to microbes than spruce. It is also likely that there were differences in either the root activities per unit of root or in the quality of the root exudates of pine, spruce and birch, as not all effects were in straight relationship with the amount of roots. In further studies comparing tree species effects, attention must be given to separating the effects of different sized root systems from differences in root activities and substrate quality. Roots not only supported microbes but also competed with them. When C supply was not limiting for microbes in the rhizosphere, there was competition for limited N between the plant and the microbes.

Acknowledgments

We thank Kimmo Valkama for counting mycorrhizas, Tuulikki Parviainen for scanning the roots, and the staff at Ruotsinkylä field station for taking good care of the plants. Dr Tuula Aarnio, Jussi Heinonsalo, Laura Paavolainen and Taina Pennanen kindly gave comments on the manuscript. We also thank Riitta Heinonen for advice in the statistical analyses. We are grateful for the Academy of Finland for supporting this work financially. Dr Karen Sims is thanked for revising the language.

References

Anderson J P E and Domsch K H 1978 A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem. 10, 215–221.

Berg B and Wessén B 1984 Changes in organic-chemical components and ingrowth of fungal mycelium in decomposing birch leaf litter as compared to pine needles. Pedobiol. 26, 285–298.

Bermingham S, Maltby L and Cooke R C 1995 A critical assessment of the validity of ergosterol as an indicator of fungal biomass. Mycol. Res. 99, 479–484.

Bradley R L and Fyles J W 1995 Growth of paper birch (*Betula papyrifera*) seedlings increases soil available C and microbial acquisition of soil-nutrients. Soil Biol. Biochem. 27, 1565–1571.

Brookes P C, Landman A, Pruden G and Jenkinson D S 1985 Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil. Biol. Biochem. 17, 837–842.

de la Rosa T M, Aphalo P J and Lehto T 1998 Effects of far-red light on the growth, mycorrhizas and mineral nutrition of Scots pine seedlings. Plant and Soil 201, 17–25.

Flaig H and Mohr H 1992 Assimilation of nitrate and ammonium by the Scots pine (*Pinus sylvestris*) seedling under conditions of high nitrogen supply. Physiol. Plant. 84, 568–576.

Grayston S J, Vaughan D and Jones D 1996 Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl. Soil Ecol. 5, 29–56.

Halonen O, Tulkki H and Derome J 1983 Nutrient analysis methods. Metsäntutkimuslaitoksen tiedonantoja 121, 28 p.

Jenkinson D S 1988 Determination of microbial biomass carbon and nitrogen in soil. *In* Advances in nitrogen cycling in agricultural ecosystems. Ed. J R Wilson. pp 368–386. CAB International, Wallingford, UK.

Joergensen R G, Anderson T-H and Wolters V 1995 Carbon and nitrogen relationships in the microbial biomass of soils in beech (*Fagus sylvatica* L.) forests. Biol. Fertil. Soils 19, 141–147.

Jukka L (ed.) 1988 Metsänterveysopas. Metsätuhot ja niiden torjunta. [Forest health guide. Forest damaging agents and their control]. Samerka Oy, Vaasa. 168 p.

Kubin E and Siira J 1980 On the suitability of the phenyl hypochlorite procedure for the determination of total nitrogen from plant and soil samples. Aquilo Ser. Bot. 17, 11–17.

Leikola M 1977 Maanmuokkaus ja pintakasvillisuuden torjunta peltojen metsittämisessä. Summary: Soil tilling and weed control in afforestation of abandoned fields. Commun. Inst. For. Fenn. 88. 3, 101 p.

Marschner H, Häussling M and George E 1991 Ammonium and nitrate uptake rates and rhizosphere pH in non-mycorrhizal roots of Norway spruce (*Picea abies* (L.) Karst.). Trees 5, 14–21.

McFee W W and Stone E L Jr 1968 Ammonium and nitrate as nitrogen sources for *Pinus radiata* and *Picea glauca*. Soil Sci. Soc. Am. Proc. 32, 879–884.

Mikola P 1948 On the physiology and ecology of *Cenococcum graniforme* especially as a mycorrhizal fungus of birch. Commun. Inst. For. Fenn. 36.3, 1–101.

Mikola P 1985 The effect of tree species on the biological properties of forest soil. Nat. Swed. Env. Prot. Board 3017, 27p.

Miles J and Young W F 1980 The effects on heathland and moorland soils in Scotland and northern England following colonization by birch (*Betula* spp.). Bull. Ecol. (France) 11, 233–242.

Moraghan JT and Buresh R 1977 Correction for dissolved nitrous oxide in nitrogen studies. Soil Sci. Soc. Am. J. 41, 1201–1202.

Norton J M and Firestone M K 1996 N dynamics in the rhizosphere of *Pinus ponderosa* seedlings. Soil Biol. Biochem. 28, 351–362.

Nykvist N 1963 Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter. Stud. For. Suec. 3, 31 p.

Nylund J-E and Wallander H 1992 Ergosterol analysis as a means of quantifying mycorrhizal biomass. Meth. Microb. 24, 77–88.

Olsson P A, Bååth E, Jakobsen I and Søderstrdm B 1996 Soil bacteria respond to presence of roots but not to mycelium of arbuscular mycorrhizal fungi. Soil Biol. Biochem. 28, 463–470. ö

Parmelee R W, Ehrenfeld J G and Tate III R L 1993 Effects of pine roots on microorganisms, fauna, and nitrogen availability in two soil horizons of a coniferous forest spodosol. Biol. Fertil. Soils 15, 113–119.

Priha O and Smolander A 1997 Microbial biomass and activity in soil and litter under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at originally similar field afforestation sites. Biol. Fertil. Soils 24, 45–51.

Ranger J and Nys C 1994 The effect of spruce (*Picea abies* Karst.) on soil development: an analytical and experimental approach. Eur. J. Soil Sci. 45, 193–204.

Ranta E, Rita H, and Kouki J 1989 Biometria, 2nd edn. Yliopistopaino, Helsinki. 569 p.

Rikala R and Huurinainen 51990 Lannoituksen vaikutus kaksivuotisten männyn paakkutaimien kasvuun taimitarhalla ja istutuksen jälkeen. Summary: Effect of fertilization on the nursery growth and outplanting success of two-year-old containerized Scots pine seedlings. Folia For. 745, 16 p.

Saarsalmi A, Palmgren K and Levula T 1992 Harmaalepän ja rauduskoivun biomassan tuotos ja ravinteiden käyttö energiapuviljelmällä. Summary: Biomass production and nutrient consumption of *Alnus incana* and *Betula pendula* in energy forestry. Folia For. 797, 29 p.

Smith 1976 Character and significance of forest tree root exudates. Ecol. 57, 324–331.

Sparling G P, Feltham C W, Reynolds J, West A W and Singleton P 1990 Estimation of soil microbial C by a fumigation-extraction method: use on soils of high organic matter content, and a reassessment of the k_{ec}-factor. Soil. Biol. Biochem. 22, 301–307.

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

West A W, and Sparling G P 1986 Modifications to the substrate induced respiration method to permit measurement of microbial biomass in soils of differing water contents. J. Microb. Meth. 5, 177–189.

Wilcox 1964 Xylem in roots of Pinus resinosa Ait. in relation to heterorhizy and growth activity. *In* The formation of wood in forest trees. Ed. M H Zimmermann. pp 459–478. Academic Press, New York.

Section editor: ??? Dr. M.D. Jones

Paper V

Priha O., Hallantie T. & Smolander A. Comparing microbial biomass, denitrification enzyme activity, and numbers of nitrifiers in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings with microscale methods. Biology and Fertility of Soils, accepted.

V

Statement of the contribution of the individual authors in paper entitled

Comparing microbial biomass, denitrification enzyme activity, and numbers of nitrifiers in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings with microscale methods

by

Outi Priha, Terhi Hallantie* and Aino Smolander

Finnish Forest Reasearch Institute, P.O.Box 18, FIN-01301 Vantaa, Finland
*Current address: Dept. of Applied Chemistry and Microbiology, P.O.Box 56 (Biocenter 1), FIN-00014 University of Helsinki. Finland

Which has been submitted to Biology and Fertility of Soils.

Contributions of the authors listed are:

Outi Priha: Corresponding author. Planning and establishing the pot experiment. Performing part of the experimental work. Responsible for writing and interpretation of the results.
Terhi Hallantie: Performing part of the experimental work
Aino Smolander: Supervisor of the Ph.D. studies of O. Priha.

All authors have given their comments of the paper before submission.

Outi Priha

Terhi Hallantie

Aino Smolander

Biology and Fertility of Soils, accepted

Outi Priha • Terhi Hallantie •Aino Smolander

Comparing microbial biomass, denitrification enzyme activity, and numbers of nitrifiers in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings with microscale methods

Abstract Flushes of C and N from fumigation-extraction (FE-C and FE-N), substrate-induced respiration (SIR), denitrification enzyme activity (DEA) and numbers of ammonium and nitrite oxidizers were studied in the rhizospheres of Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.) and silver birch (*Betula pendula* Roth) seedlings growing in soil from a field afforestation site. Rhizosphere soil was defined as the soil adhering to the roots when they were carefully separated from the rest of the soil in the pots, termed as "planted bulk soil". Soil in unplanted pots was used as control soil. All seedlings had been grown from seeds and had been infected by the natural mycorrhizas of soil. Overall, roots of all tree species tended to increase FE-C, FE-N, SIR and DEA compared to the unplanted soil, and the increase was higher in the rhizosphere soil than in the planted bulk soil. In the rhizosphere soils tree species did not differ in their effect on FE-C, FE-N and DEA, but SIR was lowest under spruce. In the planted bulk soils FE-C and SIR were lowest under spruce. The planted bulk soils differed probably because the roots of spruce did not extend as far in the pot as those of pine and birch. The numbers of both ammonium and nitrite oxidizers, determined by the most probable number method, were either unaffected or decreased by roots, with the exception of spruce rhizosphere, where numbers of both were increased.

Key words Tree species • Rhizosphere • Fumigation-extraction • Substrate-induced respiration • Denitrification enzyme activity • Autotrophic nitrifiers

Outi Priha (corresponding author) • Terhi Hallantie* • Aino Smolander
Finnish Forest Research Institute, P.O.Box 18, FIN-01301 Vantaa, Finland
Tel: +358-9-857 051, Fax: +358-9-857 2575, E-mail: Outi.Priha @metla.fi
*Current address: Dept. of Applied Chemistry and Microbiology, P.O.Box 56 (Biocenter 1), FIN-00014 University of Helsinki, Finland

Introduction

Different tree species tend to establish in different soils, but trees themselves also affect the chemical and microbial properties of soil. Spruce species have been shown to decrease the soil pH and slow down the cycling of nutrients, leading to formation of mor humus (Mikola 1985; Ranger and Nys 1994), whereas birch species may raise the pH, enhance the cycling of nutrients and lead to mull formation (Miles and Young 1980; Mikola 1985; Bradley and Fyles 1995). Trees affect the soil by their aboveground litter, by the light and temperature conditions they create in the stand, and by their root activities.

The effect of tree roots can be studied separately from the other effects of the trees in pot experiments. Microbial biomass and activities are usually higher in the rhizosphere than in bulk soil because of rhizodeposition, which leads to a larger availability of substrates in the rhizosphere (Wardle 1992). Roots and microbes can, however, also compete with each other for nutrients and water: in the rhizosphere microbes may become limited for N or other nutrients instead of C (e.g. Van Veen et al. 1989; Parmelee et al. 1993). In a pot study where Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.) and silver birch (*Betula pendula* Roth) seedlings were grown for one growing season in different soils, soil microbial biomass C and N, and C mineralization were increased by roots of pine and birch, but not by spruce roots (Priha et al. 1998). The pine root systems were about five- and birch root systems about six-fold longer than the spruce root systems, had more root tips than spruce, and also more mycorrhizal infections. As all of the soil from each pot was analyzed, the stimulating effect of pine and birch could partly be explained by quantitative differences; more roots and root tips supplying more root exudates. There were, however, indications that not only the length/mass of roots determined the changes in microbial activities, but they were also due to differences in the quality of root exudates or in the uptake mechanisms of nutrients and water by roots and mycorrhizas of these tree species (Priha et al. 1998).

In this study the aim was to study the effect of roots of these seedlings on soil microbes, without the amount of roots affecting the results. Soils under seedlings of pine, spruce and birch were studied after two growing seasons using microscale methods capable of separating the immediate rhizosphere from the "planted bulk soil", presented by Jensen and Sørensen (1994). Flushes of C and N from fumigation-extraction (FE) of the soils, substrate-induced respiration (SIR), denitrification enzyme activity (DEA), and the numbers of ammonium and nitrite oxidizers were determined.

Materials and methods

Pot experiment

The pot experiment is described in detail in Priha et al. (1998). Briefly, seeds of Scots pine, Norway spruce and silver birch were sown into 350 ml pots, containing a mixture of soil (150 ml) and gravel (150 ml), in May 1995. Altogether there were 12 treatments: pine, spruce and birch growing in pine, spruce and birch soil, and all three soils without seedlings. The soils had been taken from a 25-year-old field afforestation experiment on former agricultural land, established by Leikola (1977). In the greenhouse, the pots were divided into five blocks. The first harvest was done after one growing season, in September 1995, the results of which are presented in Priha et al. (1998).

In July 1996, seedlings were harvested for this more detailed rhizosphere study. Only seedlings growing in birch soil were used. Five pots of each treatment (pine, spruce, birch, no seedling), one from each block, were harvested.

Isolation of the rhizosphere soil and preparation of the samples

In the laboratory the soil was knocked out of the pot, and the seedlings were shaken to remove the soil from the roots. The soil adhering to the roots was defined as the rhizosphere soil and the rest as planted bulk soil. The whole root system was then transferred to a 120-ml glass bottle containing 60 ml of distilled H_2O. The bottle was vortexed for 5 s, sonicated mildly in a waterbath for 60 s and vortexed again for 10 s. Pine and birch roots were washed with another 60 ml of water, as their root systems were so much larger than those of spruce that there was more soil to be removed.

Subsequently, the root system was removed from the bottle and the solution was divided into four (spruce) or eight (pine and birch) 10-ml Vacutainer glass vials (Becton-Dickinson), one 50-ml glass tube and one crucible. The vials were for FE, SIR and DEA measurements, which were done with two replicates with all except spruce rhizosphere soil. The glass tube was for enumeration of ammonium and nitrite oxidizers, and the crucible for determination of the organic matter content of the soils. For the bulk soils (planted and unplanted), 10 g fresh weight of soil was weighed into two 120-ml glass bottles with 60 ml of distilled water, which were then treated in a similar manner as with rhizosphere soil and the solution divided into eight Vacutainer vials, one 50-ml glass tube and one crucible. Subsequently all the vials and tubes were centrifuged at 3000 rpm (1000 *g*) for 10 min, and the supernatant was discarded. The vials and tubes were then weighed in order to find out the fresh weight of the soils. They were kept in a cold room (+4°C) before the analyses. After the analyses, all the vials were dried at 105°C for 48 h for determination of the dry matter content of the samples, which was, on average, 0.2 g d.m. (dry matter).

Soil organic matter content was measured as loss-on-ignition from the dried (105°C 18 h) soil samples at 550°C for 4 h.

Seedlings

The roots were frozen in water and subsequently their length was measured by scanning them with the Mac/WinRHIZO V3.0.2 program (1995). After scanning the roots were dried at 60°C for 48 h and weighed. The shoots were dried at 60°C for 48 h and weighed.

Fumigation-extraction (FE)

FE determination was based closely on the method of Jensen and Sørensen (1994). To fumigate samples, 100 µl of ethanol-free chloroform was added directly to the vials. The vials were capped tightly and kept at 25°C for 24 h. The non-fumigated samples were kept at 4°C. After 24 h, the chloroform was evacuated using water suction. Both fumigated and non-fumigated samples were extracted with 8 ml 0.5 *M* K_2SO_4 (30 min, 200 rpm). The samples were then centrifuged at 1500 rpm (300 *g*) for 5 min, and the supernatant was filtered through a 0.45 µm membrane filter (Ion Acrodisc, Gelman Sciences). Total organic C was determined from the extracts with a total organic carbon analyzer (TOC-5000, Shimadzu) using potassium biphthalate as a standard. Total N was determined colorimetrically with a flow injection analyzer (FIAstar 5012 Analyzer + 5042 Detector, Tecator).

Substrate-induced respiration (SIR)

SIR was essentially determined according to Jensen and Sørensen (1994). The samples were conditioned at room temperature overnight. 150-200 µl glucose-water solution was added to the vials, giving 6 mg glucose per g fresh weight of soil (found to be optimal in preliminary experiments). After glucose addition, the samples were allowed to stabilize for 0.5 h. The vials were then aerated, closed with rubber stoppers and incubated at 22°C for 2 h in a rotary shaker (250 rpm). Prior to sampling, the vials were vortexed for 30 s to ensure equilibrium between gaseous and dissolved CO_2. The CO_2 evolved was measured by a gas chromatograph (Hewlett Packard 6890 Series) equipped with a thermal conductivity detector and a Megapore GS-Q column (J&W Scientific), 30 m in length, using He (5 ml min^{-1}) as carrier gas. The temperatures of the detector, injector and oven were 150, 120 and 30°C, respectively.

In a preliminary experiment, traditional SIR and the suspension SIR described above were compared. The SIR values obtained with the two methods did not differ significantly from each other (results not shown).

Denitrification enzyme activity (DEA)

DEA was measured as N_2O production with added NO_3^- and glucose as described in Priha and Smolander (1999). Briefly, 150-200 µl KNO_3-solution and 150-200 µl glucose solution were added to the vials, giving 25 µg NO_3^--N and 6 mg glucose per g fresh weight of soil (found to be optimal in preliminary experiments). The vials were filled with N_2 and a 10 kPa partial pressure of C_2H_2. The N_2O evolved was measured with a gas chromatograph after 5-h-incubation in the dark at 22°C.

Enumeration of autotrophic nitrifiers

The numbers of autotrophic nitrifiers (ammonium and nitrite oxidizers) were estimated by the most probable number (MPN) method, as described in Priha and Smolander (1999). Briefly, 18 ml of water was added to the samples, which contained after centrifugation approx. 2 g fresh weight of soil. Tenfold dilutions of the suspensions were made, and MPN tubes containing 3 ml of medium were inoculated with 1 ml of the diluted suspensions. Inoculated MPN-tubes and control tubes were incubated at room temperature (22°C) in the dark for 10 weeks.

Statistical analyses

To evaluate the overall effect of roots on microbes the rhizosphere values were compared with the values from the unplanted pots by analysis of variance using tree species and block as main effects and tree species x block as the error term (Milliken and Johnson 1984). Significant ($p \leq 0.05$) differences of the means were separated by Dunnett's test, using the unplanted soil as a control. The results were log transformed when necessary to fulfill the assumptions of variance analysis.

For tree species comparisons, the values from pots without plants were subtracted from both rhizosphere and planted bulk soil values of pots with plants in the respective blocks. The subtractions were compared by analysis of variance using a split-plot design. Tree species was the whole plot and rhizosphere (rhizosphere soil / planted bulk soil) the subplot treatment. Block was included as one treatment. The interaction between tree species and rhizosphere was also tested. Significant ($p \leq 0.05$) differences of the means were tested using Bonferroni's method. When the interaction between the tree species and rhizosphere was significant, the rhizosphere soil and planted bulk soil were compared within one tree species, the rhizosphere soils of pine, spruce and birch were compared against each other, and the planted bulk soils of pine, spruce and birch were compared against each other.

Results

Roots

Both the length and the dry mass of the roots were significantly ($p < 0.01$) lower in spruce seedlings as compared to pine and birch seedlings (Table 1). Root weight ratio (root weight / total weight of the seedling) was significantly ($p < 0.01$) lower under pine than under spruce and birch.

Table 1. Root weight, root weight ratio (root weight / total weight of the seedling) and length of the roots. Values are means of five seedlings, standard errors of the means in parentheses.

Tree species	Root weight, mg d.m.	Root weight ratio	Root length, cm
Pine	690 (50)	0.26 (0.01)	1460 (110)
Spruce	180 (40)	0.43 (0.05)	450 (90)
Birch	890 (170)	0.41 (0.02)	1610 (180)

d.m. = dry matter

Organic matter content of soils

The soil organic matter content varied from 12.7 to 14.8% of d.m. (results not shown). Statistically significant differences were not observed.

Flushes of C and N from fumigation-extraction (FE)

For all tree species, roots had significantly ($p = 0.04$) increased FE-C (Fig. 1a). The interaction between tree species and rhizosphere was statistically significant for the subtracted values of FE-C (Table 2). Within spruce, FE-C was higher in the rhizosphere than in planted bulk soil, but within pine and birch rhizosphere soil and planted bulk soil did not differ from each other. The rhizosphere soils of different tree species did not differ from each other, but in the planted bulk soils FE-C was lower under spruce than under birch.

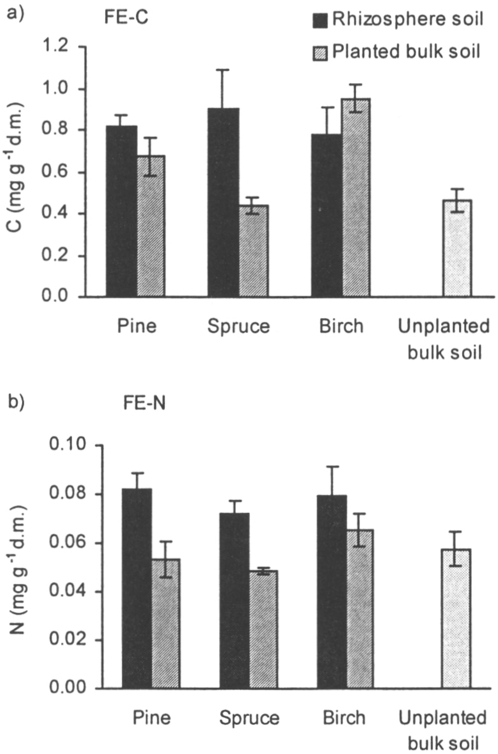


Fig. 1. The flush of a) extractable C and b) extractable N from fumigation of the soils. Values are means of five pots, bars show standard errors of the means.

Roots had increased FE-N for all the tree species, but the effect was not statistically significant ($p = 0.12$) (Fig. 1b). The subtracted values of FE-N were not affected by the tree species, but were significantly higher in the rhizosphere soils than in planted bulk soils within all the tree species (Table 2).

Substrate-induced respiration (SIR)

Roots of all tree species had significantly ($p < 0.01$) increased SIR (Fig. 2). The interaction between tree species and rhizosphere was significant for the subtracted values of SIR (Table 2). Under pine and spruce, the SIR was higher in the rhizosphere soil than in planted bulk soil. The SIR was lower in spruce rhizosphere than in pine rhizosphere and lower in planted bulk soil of spruce than that of birch.

Denitrification enzyme activity (DEA)

Roots of all tree species had increased DEAs, but the increase was not statistically significant ($p = 0.15$) (Fig. 3). The subtracted DEA values tended to be higher in the rhizosphere soils than in planted bulk soils, but the differences were not statistically significant (Table 2). Tree species did not affect the DEA differently.

Table 2. Flushes of C and N from fumigation (FE-C and FE-N), substrate-induced respiration (SIR), denitrification enzyme activity (DEA), and most probable numbers (MPN) of ammonium and nitrite oxidizers in the soils. Values from pots without plants subtracted from both rhizosphere and planted bulk soil values of pots with plants in respective blocks.
Values are means of five pots, standard errors of the means in parentheses.

Tree species	**Soil**		**FE-C, mg g^{-1} d.m.**	**FE-N, µg g^{-1} d.m.**	**SIR CO_2-C, µg g^{-1} d.m. h^{-1}**	**DEA N_2O-N, µg g^{-1} d.m. h^{-1}**	**NH_4^+-oxidizers, MPN g^{-1} d.m.**	**NO_2^--oxidizers, MPN g^{-1} d.m.**
Pine	Rhizosphere soil		0.35 (0.08)	24.3 (6.0)	21.7 (5.3)	0.12 (0.15)	-71 000 (25 000)	-27 000 (12 000)
	Planted bulk soil		0.21 (0.09)	-4.0 (2.0)	8.0 (2.4)	0.06 (0.11)	-8 000 (44 000)	-16 000 (9 000)
Spruce	Rhizosphere soil		0.44 (0.21)	14.4 (8.0)	7.2 (4.1)	0.28 (0.02)	324 000 (131 000)	12 000 (19 000)
	Planted bulk soil		-0.02 (0.03)	-8.9 (8.0)	-0.8 (2.0)	-0.01 (0.15)	47 000 (35 000)	-5 000 (10 000)
Birch	Rhizosphere soil		0.32 (0.16)	21.6 (17.0)	16.4 (5.7)	0.30 (0.08)	-71 000 (25 000)	-26 000 (7 000)
	Planted bulk soil		0.49 (0.11)	7.7 (8.0)	13.7 (5.0)	0.10 (0.11)	7 000 (71 000)	-11 000 (11 000)
Anova	**Source of variation**	**df**			***p*-value**			
	Tree species	2	0.17	0.50	0.00	0.47	0.07	0.04
	Rhizosphere	1	0.11	0.00	0.00	0.07	0.21	0.19
	Tree species x rhizosphere	2	0.02	0.46	0.02	0.59	0.03	0.05

d.m. = dry matter

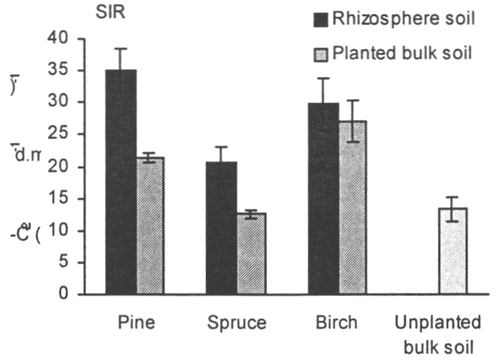


Fig. 2. CO_2-C evolved from substrate-induced respiration of the soils. Values are means of five pots, bars show standard errors of the means.

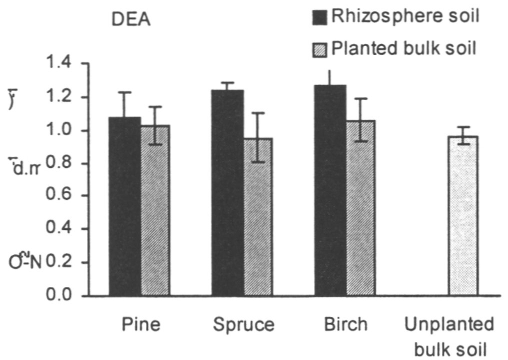


Fig. 3. Denitrification enzyme activity of the soils. Values are means of five pots, bars show standard errors of the means.

Numbers of nitrifiers

Both ammonium and nitrite oxidizers were decreased by roots of pine and birch, but increased by roots of spruce (Fig. 4). For subtracted values of both ammonium and nitrite oxidizers, the interaction between tree species and roots was significant (Table 2). Under pine and birch, the numbers of ammonium oxidizers, and under birch also the numbers of nitrite oxidizers, were lower in rhizosphere soil than in planted bulk soil. Under spruce, the numbers of both ammonium and nitrite oxidizers were higher in rhizosphere soil than in planted bulk soil. There were more both ammonium and nitrite oxidizers in spruce rhizosphere soil than in pine and birch rhizosphere soils. The numbers in planted bulk soils did not differ from each other under different tree species.

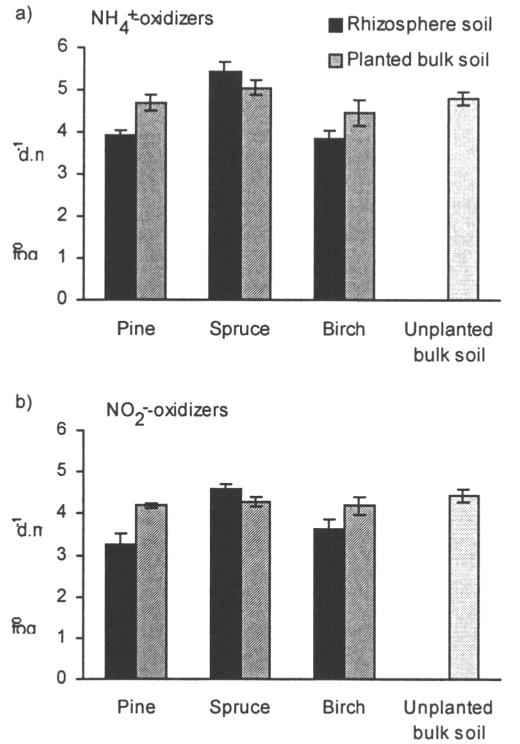


Fig. 4. Numbers of a) ammonium and b) nitrite oxidizers in the soil, measured by the most probable number (MPN) method. Values are means of five pots, bars show standard errors of the means.

Discussion

As expected, FE-C and SIR were increased by roots (Figs 1a and 2, Table 2). In the rhizosphere soils FE-C was at the same level for all three tree species. In planted bulk soils, both FE-C and SIR tended to be higher in birch and pine soil than in spruce soil, where both were at the same level as in the unplanted soil, which is in accordance with our earlier results (Priha et al. 1998). This suggests that the increasing effect of pine and birch roots on microbial biomass C was very much due to their higher amount of roots (Table 1). In the rhizosphere all tree species had the same effect, but the planted bulk soil was in closer proximity to the roots of pine and birch than those of spruce. The rhizosphere effect is a gradient from roots, and in this study the rhizosphere samples of pine, spruce and birch were comparable, but the planted bulk soil was further in the gradient with spruce than with pine and birch.

The rooting densities of pine, spruce and birch at field conditions, especially at soils of the same fertility, are not well known. There are some results, however, indicating that the same differences in the rooting densities as found in the pot experiment may occur in the field. Kalela (1949) compared horizontal root systems of spruce and pine of the same size or of the same age, and found that throughout early stages, pine always had larger root systems than spruce. At the age of 110 years and cubic volume of 0.35 m^3, trees had root systems of approximately the same size, but after that the root systems of spruce were larger.

It is likely that it is not only the roots of pine and birch that extend further than those of spruce, but also the extramatrical mycelium of their mycorrhizas. All seedlings were mycorrhizal, and the amount of ergosterol, an indicator of fungal biomass, was higher under pine and birch, suggesting that they had more extramatrical mycelium in soil than spruce (Priha et al. 1998). Although we did not focus on mycorrhizas in this study, their extramatrical hyphae are included in the FE-C and FE-N measurements. In addition to their direct inclusion in soil microbial biomass, mycorrhizas can also change the soil microbial biomass indirectly by affecting the bacterial communities in soil. It has been suggested that the external mycorrhizal mycelium distributes plant C to soil compartments beyond the rhizosphere (Hobbie 1992), and different ectomycorrhizal fungi have been shown to change bacterial community structure in the mycorrhizosphere (Timonen et al. 1998; Olsson 1998).

In addition to the amount and extension of roots and mycorrhizas, root activities per unit of root differed between species, as indicated by the lower SIR in spruce rhizosphere (Fig. 2, Table 2). The nutrient and water uptake mechanisms and respiration rate of roots can differ, and these processes affect the surrounding soil profoundly. Further, not only the quantity, but also the quality of the root exudates may differ between species; the root exudates of pine and birch could be better substrates for microbes than those of spruce (reviewed by Grayston et al. 1996). The fact that only SIR was lower in spruce rhizosphere, and not FE-C and FE-N, suggests that pine and birch stimulate the active part of the microbial population.

Earlier results showed that there was competition for N between microbes and seedlings in the pots (Priha et al. 1998). Compared with FE-N values from the first harvest, the values in planted bulk soils were on the same level, but FE-N in the rhizosphere soil of all the seedlings was higher (Fig. 1b). The extra N in microbes in the rhizosphere may have been caused by the extra C surplus helping microbes in the mineralisation of native N from soil (priming effect). Studies with glucose-amended soils, however, suggest that bacteria do not mineralize organic N when given a surplus of C (Elliott et al. 1983). The seedlings may also have provided root exudates containing N, as root exudates of trees have been shown to contain amino acids (Grayston et al. 1996).

The roots of all the tree species tended to increase DEA, but the variation in DEA was considerable, and the increase was not statistically significant (Fig. 3, Table 2). Increased denitrification in the rhizosphere would be plausible, as it has been shown with annual plants that denitrification often increases in the rhizosphere, because of the extra C surplus and anaerobic conditions caused by root respiration (Wollersheim et al. 1987; Wheatley et al. 1990). DEA was not differently affected by the tree species. Thus, it can be hypothesized that the oxygen and C situations in the rhizospheres of pine, spruce and birch were not strikingly different.

The numbers of ammonium oxidizers have been shown to reflect reasonably well the changes in potential nitrification activity of soil (Martikainen 1985; Aarnio and Martikainen 1996). Within birch and pine there was a slightly decreasing effect of the rhizosphere on the numbers of nitrifiers (Fig. 4, Table 2), which is in accordance with earlier studies (Wheatley et al. 1990). The numbers of both ammonium and nitrite oxidizers were, however, increased in spruce rhizosphere. Whether this difference between tree species was due to differences in pH or availability of ammonium, is not known.

In conclusion, it seems that the increasing effect of roots of pine and birch, as compared to spruce, on the soil microbial biomass C was mostly caused by their higher rooting densities and root and mycorrhizal extension, because microbial biomass C did not differ in the rhizospheres of these seedlings, but differed in the planted bulk soils. SIR was lower in spruce rhizosphere than in pine and birch rhizospheres, suggesting that pine and birch have stimulated the active part of microbial population. There were differences in the effect of tree species on nitrifier populations: the numbers of nitrifiers were either unaffected or decreased by pine and birch roots, but were increased by spruce roots.

Acknowledgements We thank Tuulikki Parviainen for scanning the roots, and the staff at Ruotsinkylä field station for taking good care of the plants. We are grateful to Risto Häkkinen for advice in the statistical analyses, and to Dr. Tuula Aarnio, Jussi Heinonsalo and Laura Paavolainen for comments on the manuscript. Donald Smart is thanked for revising the language. We also thank the Academy of Finland for supporting this work financially.

References

Aarnio T, Martikainen PJ (1996) Mineralization of carbon and nitrogen, and nitrification in Scots pine forest soil treated with fast- and slow-release nitrogen fertilizers. Biol Fertil Soils 22:214-220

Bradley RL, Fyles JW (1995) Growth of paper birch (*Betula papyrifera*) seedlings increases soil available C and microbial acquisition of soil-nutrients. Soil Biol Biochem 27:1565-1571

Elliott ET, Cole CV, Fairbanks BC, Woods LE, Bryant RJ, Coleman DC (1983) Short-term bacterial growth, nutrient uptake, and ATP turnover in sterilized, inoculated and C-amended soil: The influence of N availability. Soil Biol Biochem 15:85-92

Grayston SJ, Vaughan D, Jones D (1996) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl Soil Ecol 5:29-56

Hobbie SE (1997) Effects of plant species on nutrient cycling. TREE 7:336-339

Jensen LS, Sørensen J (1994) Microscale fumigation-extraction and substrate-induced respiration methods for measuring microbial biomass in barley rhizosphere. Plant and Soil 162:151-161

Kalela EK (1949) Männiköiden ja kuusikoiden juurisuhteista I. Summary: On the horizontal roots in pine and spruce stand I. Acta For Fenn 57:1-79

Leikola M (1977) Maanmuokkaus ja pintakasvillisuuden torjunta peltojen metsittämisessä. Summary: Soil tilling and weed control in afforestation of abandoned fields. Commun Inst For Fenn 88:1-101

Martikainen PJ (1985) Numbers of autotrophic nitrifiers and nitrification in fertilized forest soil. Soil Biol Biochem 17:245-248

Mikola P (1985) The effect of tree species on the biological properties of forest soil. Nat Swed Env Prot Board 3017:1-29

Miles J, Young WF (1980) The effects on heathland and moorland soils in Scotland and northern England following colonization by birch (*Betula* spp.). Bull Ecol (France) 11:233-242

Milliken GA, Johnson DE (1984) Analysis of messy data. Van Nostrand Reinhold Company, New York. 473 p.

Olsson PA (1998) The external mycorrhizal mycelium. PhD thesis, Department of Ecology, Lund University, Sweden.

Parmelee RW, Ehrenfeld JG, Tate RL III (1993) Effects of pine roots on microorganisms, fauna, and nitrogen availability in two soil horizons of a coniferous forest spodosol. Biol Fertil Soils 15:113-119

Priha O, Lehto T, Smolander A (1998) Mycorrhizas and C and N transformations in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings. Plant and Soil 206: 191-204

Priha O and Smolander A (1999) Nitrogen transformations in soil under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. Soil Biol Biochem 31: 965-977 (in press)

Ranger J, Nys C (1994) The effect of spruce (*Picea abies* Karst.) on soil development: an analytical and experimental approach. Eur J Soil Sci 45:193-204

Timonen S, Jørgensen KS, Haahtela K, Sen R (1998) Bacterial community structure at defined locations of *Pinus sylvestris-Suillus bovinus* and *-Paxillus involutus* mycorrhizospheres in dry pine forest humus and nursery peat. Can J Microbiol 44:499-513

Van Veen JA, Merckx R, Van de Geijn SC (1989) Plant- and soil related controls of the flow of carbon from roots through the soil microbial biomass. Plant and Soil 115:179-188

Wardle DA (1992) A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol Rev 67:321-358

Wheatley R, Ritz K, Griffiths B (1990) Microbial biomass and mineral N transformations in soil planted with barley, ryegrass, pea or turnip. Plant and Soil 127:157-167

Wollersheim R, Trolldenier G, Beringer H (1987) Effect of bulk density and soil water tension on denitrification in the rhizosphere of spring wheat (*Triticum vulgare*). Biol Fertil Soils 5:181-187

Paper VI

Priha O., Grayston S.J., Pennanen T. & Smolander A. Microbial activities related to C and N cycling, and microbial community structure in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings in an organic and mineral soil. Submitted manuscript.

VI

Statement of the controbutors of the individual authors in paper entitled

Microbial activities related to C and N cycling, and microbial community structure in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings in an organic and mineral soil

by

Outi Priha, Susan J. Grayston*, Taina Pennanen and Aino Smolander

Finnish Forest Reasearch Institute, P.O.Box 18, FIN-01301 Vantaa, Finland
*Macaulay Land Use Reseach Institute, Craigiebuckler, Aberdeen AB15 8QH, U.K.

Contributions of the authors listed are:

Outi Priha: Corresponding author. Planning and establishing the pot experiment. Performing experimental work. Responsible for writing and interpretation of the results.
Susan J. Grayston: Advice in performing the Biolog-measurements.
Taina Pennanen: Advice in performing phospholipid fatty acid analyses and interpreting them.
Aino Smolander: Supervisor of the Ph.D. studies of O. Priha.

All authors have given their comments of the paper before submission.

Outi Priha

Susan J. Grayston

Taina Pennanen

Aino Smolander

MICROBIAL ACTIVITIES RELATED TO C AND N CYCLING, AND MICROBIAL COMMUNITY STRUCTURE IN THE RHIZOSPHERES OF *PINUS SYLVESTRIS, PICEA ABIES* AND *BETULA PENDULA* SEEDLINGS IN AN ORGANIC AND MINERAL SOIL

Outi Priha, Susan J. Grayston*, Taina Pennanen, Aino Smolander
Finnish Forest Research Institute, P.O.Box 18, FIN-01301 Vantaa, Finland
*Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen AB15 8QH, U.K.

Key words: Carbon mineralization - Nitrogen transformations - Microbial community structure - Scots pine - Norway spruce - Silver birch

Abstract

Seedlings of Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) Karst.) and silver birch (*Betula pendula* Roth) were planted into pots of two soils types: an organic soil and a mineral soil. Pots without seedlings were also included. After one growing season microbial biomass C (C_{mic}) and N (N_{mic}), C mineralization, net ammonification, net nitrification, denitrification potential, phospholipid fatty acid (PLFA) patterns and community level physiological profiles (CLPPs) were measured in the rhizosphere soil of the seedlings. The aim was to determine whether pine, spruce and birch seedlings have a selective influence on the soil microbial community structure and activity, and whether this varies in an organic and mineral soil.

In the organic soil C_{mic} and N_{mic} were higher in birch rhizosphere than in pine and spruce rhizosphere. C mineralization rate was not affected by tree species. Unplanted soil contained the highest amount of mineral N and birch rhizosphere the lowest, but rates of net N mineralization and net nitrification did not differ between treatments. The microbial community structure, measured by PLFAs, had changed in the rhizospheres of all tree species compared to the unplanted soil. Birch rhizosphere was most clearly separated from the others; there were more of the fungal specific fatty acid 18:2ω6,9 and more branched fatty acids, common in Gram-positive bacteria, in this soil. CLPPs, done with Biolog GN plates and 30 additional substrates, separated only birch rhizosphere from the others.

In the mineral soil roots of all tree species stimulated C mineralization in soil, and prevented nitrification, but did not affect C_{mic} and N_{mic}, PLFA patterns or CLPPs. The effects of different tree species did not vary in the mineral soil. Thus, in the mineral soil the strongest effect on soil microbes was the presence of a plant, regardless of tree species, but in the organic soil different tree species varied in their influence on soil microbes.

Introduction

Roots of different tree species affect the surrounding soil profoundly. Usually the extra C results in increased microbial biomass and numbers in the rhizosphere [1], but plants can also compete with microbes for mineral nutrients, especially N [2,3,4]. Different microbial groups may be affected differently by roots: denitrification is often enhanced in the rhizospheres of annual plants, but nitrification may become less important [5,6]. Microbial community structure has also been shown to change in the rhizospheres of different tree species [7]. In a pot study where Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) Karst.) and silver birch (*Betula pendula* Roth) were grown from seeds for one growing season, soil microbial biomass C and N, and C mineralization rate were increased by roots of pine and birch, but not by spruce roots [8]. The stimulatory effect of pine and birch seemed to be partly due to their higher number of short roots and mycorrhizas compared to spruce, and the higher amount of labile C their roots release to soil, as suggested also by Bradley and Fyles [9] regarding paper birch (*Betula papyrifera*). Nevertheless, there can be also qualitative differences in the root activities of pine, spruce and birch.

The aim of this study was to determine whether the microbial community structure and activity are different in the rhizospheres of pine, spruce and birch, when the amount of roots does not influence the results. We also wanted to assess whether these possible effects vary in an organic and mineral soil.

Materials and methods

Experimental design

Seedlings of Scots pine, Norway spruce and silver birch were planted into pots of two soils types: an organic soil and a mineral soil. Pots without seedlings (unplanted) were also included. After one growing season the following parameters were measured from the soil: microbial biomass C and N (C_{mic} and N_{mic}), C mineralization, net ammonification, net nitrification, denitrification potential, denitrification enzyme activity, phospholipid fatty acid (PLFA) patterns, community level physiological profiles (CLPP), using Biolog plates, and plate counts of culturable microbes.

Soils

The soils were taken from a 60-year-old Scots pine forest representing a *Vaccinium vitis-idaea* (VT) -type [10]. This field experiment has been described in detail in Priha and Smolander [11]. Soil from the humus layer (organic soil) and separately from the 0-20 cm mineral soil below was collected from four areas of the plot. The soils were sieved (mesh size 6 mm for organic soil and 4 mm for mineral soil) and mixed thoroughly. The organic matter content of the organic soil was 64.8% of d.m. (dry matter) and pH($CaCl_2$) 3.4, and those of the mineral soil 5.1% and 4.1, respectively.

Seedlings

Seedlings of Southern Finnish provenance were obtained from the Suonenjoki Research Station of Finnish Forest Research Institute. We aimed at having seedlings of approximately the same size, instead of the same age, and had two-year-old pine and spruce seedlings, and one-year-old birch seedlings. The seedlings of pine, spruce and birch had, on average, a dry mass of 4.0, 3.2 and 3.6 g. The seedlings were growing in peat pots, but the roots were washed free of peat before planting. All of the seedlings were confirmed to be mycorrhizal by microscopic analysis of the roots [12].

Pot experiment
The pot experiment was started at the beginning of June, 1996. 100 ml of gravel, washed with water, was spread to the bottom of 1.5 l pots. Either 0.5 kg f.w. (fresh weight) of the organic soil or 1 kg f.w. of the mineral soil was put to the pots. The seedlings were planted and another 100 ml of washed gravel was added to the surface of the soil to prevent formation of moss and drying of the surface soil. Altogether there were 8 treatments: pine, spruce and birch growing in organic soil and in mineral soil, and both soils without seedlings. There were 20 pots per treatment.

The temperature in the greenhouse was 22°C during the day (16 h) and 18°C during the night (8 h). Na-lamps (Elektro-valo) were used during the daytime when light intensity decreased below 200 W m^{-2}. Seedlings were watered to saturation approximately every other day and their positions were systematically changed every two weeks.

Harvest
After three months, at the beginning of September 1996, five pots of each treatment were harvested for soil analyses. In the laboratory seedlings were removed from the pots, and the roots were shaken to remove the adhering soil. The soil which remained on the roots was considered as rhizosphere soil. An attempt was made to leave a similar layer of soil on the roots of all tree species. An additional three pots from each treatment were harvested for determining the PLFAs, CLPPs and plate counts. All of the analyses were done with fresh soil, kept at +4°C, one to two weeks from the harvest, except the PLFA analysis, for which soils were stored at -18°C.

Analyses of the seedlings
The shoots were dried at 40°C for 48 h and weighed. The needles/leaves were separated and ground and their total N concentration was measured with an automated CHN analyzer (Leco CHN-600). Roots were frozen in water and subsequently they were dried at 60°C for 48 h and weighed.

Chemical analyses of the soils
The dry matter content of the soil was determined by drying the samples at 105°C for 24 h. Soil organic matter content was measured as loss on ignition from the dried samples at 550°C for 4 h. Water-holding capacity (WHC) was measured by soaking the soil samples in water for 2 h and then draining for 2 h. Soil pH was measured in soil : 0.01 *M* $CaCl_2$ suspensions (3:5 v/v). Total organic C and total N were measured from dried (40°C) samples using an automated CHN analyzer (Leco CHN-600).

Analyses of microbial biomass and activities of C and N cycles
The measurements of C_{mic} and N_{mic} with fumigation-extraction (FE) and substrate-induced respiration (SIR) methods have been described earlier [13]. These results were shown without the use of conversion factors. Rate of C mineralization was evaluated as CO_2-C production at 14°C (basal respiration, [13]). Net ammonification and nitrification were measured by incubating the soil samples at 14°C for 40 d [13]. For all of these

measurements, duplicate 1.5 g d.m. humus and 6 g d.m. mineral soil samples with the soil moisture content adjusted to 60% of the WHC were used.

The numbers of autotrophic nitrifiers (ammonium and nitrite oxidizers) were estimated by the most probable number (MPN) method, as described in [11]. The tubes were incubated in the dark at room temperature (22°C) for 14 weeks.

Denitrification activity was measured as N_2O production, as described in [11]. Briefly, duplicate 1.5 g d.m. humus samples or 6 g d.m. mineral soil samples with soil moisture content adjusted to 100% of the WHC were incubated at 14°C under 10 kPa partial pressure of acetylene. Results given are production rates of N_2O-N between 1 and 2 days. Denitrification enzyme activity (DEA) aims at determining the activity of pre-existing denitrifying enzymes in soil, without allowing denitrifying organisms to proliferate [14]. DEA was measured with the same amounts of soil as above, but adding solutions of KNO_3 and glucose [11]. The samples were incubated for approximately 5 h in the dark at 22°C and N_2O was measured.

PLFA analysis
The phospholipid extraction and analysis of PLFAs was conducted as described by Frostegård *et al.* [15]. Briefly, 0.5 g f.w. (fresh weight) organic soil and 2.5 g f.w. mineral soil samples were extracted with a chloroform:methanol:citrate buffer -mixture (1:2:0.8), and the lipids were separated into neutral lipids, glycolipids, and phospholipids in a silicic acid column. The phospholipids were subjected to mild alkaline methanolysis, and the fatty acid methyl esters were separated by gas chromatography (Hewlett Packard 5890) equipped with a flame ionization detector and a HP-5 (phenylmethyl silicone) capillary column, 50 m in length, using He as a carrier gas. Peak areas were quantified by adding methyl nonadecanoate fatty acid (19:0) as an internal standard.

Fatty acids are designated in terms of total number of carbon atoms : number of double bonds, followed by the position of the double bond from the methyl end of the molecule. The prefixes a, i and br indicate anteiso, iso and unknown branching, respectively. The prefix cy indicates a cyclopropane fatty acid, and methyl branching (Me) is indicated as the position of the methyl group from the carboxyl end of the chain. The prefix C (C15:1) indicates that the PLFA has 15 carbon atoms and one double bond, but the arrangement of the carbon atoms (e.g. branching position) is not confirmed.

The sum of PLFAs considered to be mainly of bacterial origin (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) was chosen to represent bacterial biomass (bacterial PLFAs) [16]. The quantity of 18:2ω6,9 was used as an indicator of fungal biomass (fungal PLFA). The ratio fungal PLFA to bacterial PLFAs was also calculated.

CLPPs
Community level physiological profiles (CLPPs) were conducted using

Biolog plates, according to Campbell *et al.* [17]. Briefly, to extract the microbes, soil samples (10 g f.w.) were shaken in 100 ml 1/4 strength Ringers solution (Oxoid) for 10 min on an orbital shaker. The 10^{-4} dilution of each soil sample was centrifuged at 750 *g* for 10 min to remove soil and root particles which might introduce additional C into the wells. A 150 μl aliquot of the supernatant from the centrifuged samples was added to each well of a Biolog GN plate (Biolog Inc., Hayward, CA, USA) and an MT plate with 30 additional carbon sources representing compounds reported in the literature to be plant root exudates [17]. Plates were incubated at 15°C and colour development measured as absorbance at 590 nm (A_{590}) using a microplate reader (Emax, Molecular Devices, Oxford, UK). Absorbance was measured at 0 h, then every 24 h for 5 days, and then at 10 d and 15 d.

We compared all 125 C sources with the 61 identified as being plant root exudates (30 from MT plate plus 31 from the GN plate, see [17]). When calculating the results, first the 0 h reading was subtracted from each of the wells, as some compounds were initially coloured. The blanks from both GN and MT plates were then subtracted and the average well colour development (AWCD, [18]) was calculated for each time point. In order to eliminate variation in AWCD, which may arise from different cell densities in different samples, Garland [19] recommended comparison of samples of equivalent AWCD. We used on each sample the time point at which the AWCD was closest to the value of 0.75. This time point was either 10 or 15 d for all samples. The values from different time points were then all divided by their respective AWCDs.

Plate counts

The soil samples (10g f.w.) were shaken in 100 ml ¼ strength Ringers solution (Oxoid) for 10 min on an orbital shaker. The samples were then serially diluted to 10^{-7} in ¼ strength Ringers solution and suspensions (0.1 ml) spread, in duplicate, onto the following media: Tryptone Soy agar (1/10 Oxoid strength) plus cycloheximide (50 mg l^{-1}) for enumeration of bacteria and actinomycetes; *Pseudomonas* Isolation agar (Oxoid) selective for populations of pseudomonads; Czapek Dox agar (Oxoid) + streptomycin sulphate (50 mg l^{-1}) + tetracycline hydrochloride (50 mg l^{-1}) + ampicillin (10 mg l^{-1}) for enumeration of yeasts and fungi. The plates were incubated at 25°C and colonies counted after 5-6 days and again after 13-14 days.

Statistical analyses

Means of the measured characteristics between treatments were compared with analysis of variance [20]. Organic soil and mineral soil were tested separately. Results were log or log(x+1) transformed when necessary for fulfilling the assumptions of variance analysis. Significant differences of the means were separated by Tukey's test (HSD, honestly significant difference) [20].

The mole percents of PLFA values and the Biolog values divided by AWCD were subjected to principal component analysis (PCA) using a correlation matrix [21]. PCA was done separately for organic and mineral soil samples. The Systat 6.0.1 (SPSS Inc, 1996) statistical software was used.

Results

Seedlings

Some characteristics of the seedlings at the time of harvest are shown in Table 1. The size of the seedlings did not differ substantially when growing in different soils, but the needles/leaves of all tree species had approximately two times higher N concentrations when growing in the organic soil as compared to mineral soil. N concentrations in the needles/leaves did not differ much between different tree species within one soil, but on a basis of the whole seedling, pine and birch contained significantly more N than spruce when growing in the organic soil.

Physical and chemical characteristics of the soils

There were small, but significant, differences in the soil pH between tree species (Table 1). The soil organic matter content also varied between treatments, but the concentration of total soil N did not differ significantly between treatments.

Microbial biomass C and N, substrate-induced and basal respiration

In the organic soil, the flush of C from fumigation was higher in birch rhizosphere than in pine and spruce rhizosphere, and unplanted soil did not differ statistically significantly from any of the soils (Fig. 1a). The flush of N from fumigation was highest in birch rhizosphere (Fig. 1b). In the mineral soil the flushes of C and N did not differ statistically significantly between treatments.

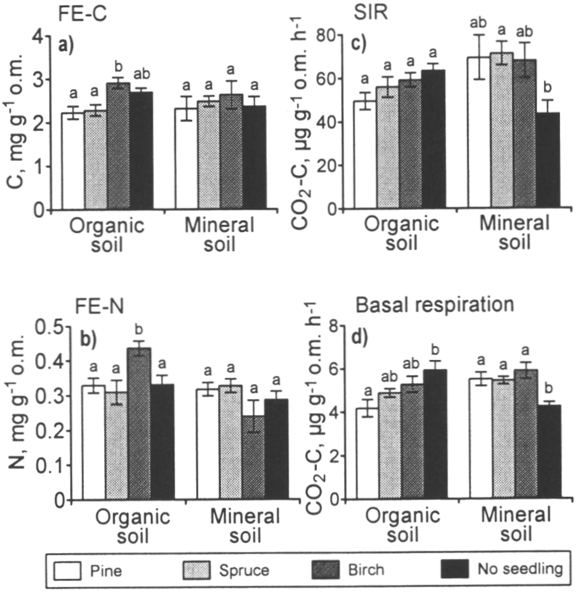


Fig. 1. The flush of a) extractable C and b) extractable N from fumigation of the soils, c) substrate-induced respiration and d) basal respiration of the soils. Values are means of five pots, bars show standard errors of the means. Values with the same letter are not significantly ($p \leq 0.05$) different from each other.

Table 1. Some characteristics of the seedlings and soils. Values are means of five pots, standard errors of the means in parentheses. Values with the same letter within one soil are not significantly ($p \leq 0.05$) different from each other.

Soil	Tree species	Shoot, cm	Shoot, g d.m.	Roots, g d.m.	N in needles/ leaves, mg g^{-1} d.m.	N in needles/ leaves of the whole plant, mg	Soil pH ($CaCl_2$)	Soil organic matter, % of d.m.	Total soil N mg g^{-1} o.m.
Organic soil	Pine	34 (1) ^{a}	6.0 (0.4) ^{a}	1.6 (0.1) ^{a}	20 (1) ^{a}	61 (5) ^{a}	3.3 (0.0) ^{a}	69 (3) ^{a}	1.9 (0.2) ^{a}
	Spruce	34 (1) ^{a}	4.1 (0.4) ^{a}	2.1 (0.3) ^{a}	23 (1) ^{b}	35 (3) ^{b}	3.4 (0.0) ^{a}	68 (3) ^{ab}	1.9 (0.1) ^{a}
	Birch	116 (6) ^{b}	13.1 (0.8) ^{b}	6.3 (0.5) ^{b}	21 (1) ^{ab}	52 (5) ^{a}	3.2 (0.0) ^{b}	59 (4) ^{ab}	2.0 (0.1) ^{a}
	No seedling						3.6 (0.0) ^{c}	55 (3) ^{b}	1.9 (0.1) ^{a}
Mineral soil	Pine	32 (1) ^{a}	5.2 (0.8) ^{a}	2.7 (0.5) ^{a}	10 (0) ^{a}	23 (4) ^{a}	4.5 (0.0) ^{a}	4.5 (0.0) ^{a}	1.8 (0.1) ^{a}
	Spruce	34 (1) ^{a}	3.9 (0.5) ^{a}	3.6 (0.6) ^{a}	12 (1) ^{a}	20 (1) ^{a}	4.2 (0.0) ^{b}	4.8 (0.1) ^{bc}	1.9 (0.1) ^{a}
	Birch	90 (3) ^{b}	10.5 (0.9) ^{b}	10.0 (1.6) ^{b}	11 (0) ^{a}	20 (2) ^{a}	4.4 (0.0) ^{c}	5.1 (0.1) ^{c}	1.9 (0.1) ^{a}
	No seedling						4.5 (0.0) ^{a}	4.7 (0.1) ^{ab}	1.8 (0.1) ^{a}

d.m. = dry matter
o.m. = organic matter

In the organic soil, SIR did not differ statistically significantly between different treatments, but in the mineral soil SIR was lowest in unplanted soil, although the difference was statistically significant only for spruce (Fig. 1c). In the organic soil, basal respiration was not significantly affected by the tree species, but was higher in unplanted soil than in pine rhizosphere (Fig. 1d). In the mineral soil basal respiration was lowest in unplanted soil, and did not differ between rhizospheres of different tree species.

Ammonification, nitrification and numbers of nitrifiers

In the organic soil, the concentration of mineral N, and both ammonium and nitrate separately, were highest in unplanted soil and lowest in birch rhizosphere (Table 2). Also in the mineral soil the concentrations were highest in pots without seedlings. All soils contained negligible or small amounts of nitrate. In the organic soil, net formation of mineral N did not differ between treatments, but in the mineral soil it was significantly greater in the spruce rhizosphere. Considerable net nitrification occurred only in the unplanted mineral soil.

In the organic soil, there were negligible numbers of ammonium oxidizers in all treatments (Fig. 2). Numbers of nitrite oxidizers tended to be lowest in birch rhizosphere. In the mineral soil, numbers of both ammonium and nitrite oxidizers were significantly higher in unplanted soil, but did not differ in the rhizospheres of different tree species.

Denitrification and denitrification enzyme activity

Denitrification activity was low in all of the soils. In the organic soil, denitrification rate was lowest and DEA highest in birch rhizosphere (Fig. 3). In the mineral soil, denitrification activity was significantly higher in unplanted soil than in the rhizosphere soils, but DEA did not differ significantly between different treatments. DEA correlated positively (r = 0.79, $p < 0.00$) with plant dry weight in the organic soils, but not in the mineral soils (r = 0.24, $p = 0.39$).

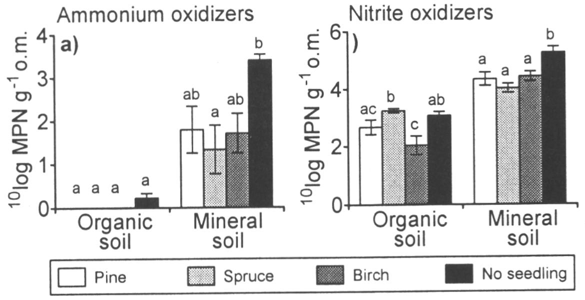


Fig. 2. Numbers of a) ammonium oxidizers and b) nitrite oxidizers determined by the most probable number method. Values are means of five pots, bars show standard errors of the means. Values with the same letter are not significantly ($p \leq 0.05$) different from each other.

Table 2. Initial concentrations of mineral N and net formation of mineral N in an aerobic incubation. Values are means of five pots, standard errors of the means in parentheses. Values with the same letter within one soil are not significantly ($p \leq 0.05$) different from each other.

Soil	**Tree species**	**Initial, μg g^{-1} o.m.**			**Net formation, μg g^{-1} o.m. 40 d^{-1}**		
		NH_4^+-N	**(NO_2^-+NO_3^-)-N**	**(NH_4^++NO_2^-+NO_3^-)-N**	**NH_4^+-N**	**(NO_2^-+NO_3^-)-N**	**(NH_4^++NO_2^-+NO_3^-)-N**
Organic soil	Pine	301 (31) ^{a}	1 (1) ^{a}	302 (31) ^{a}	238 (31) ^{a}	0 (0) ^{a}	238 (31) ^{a}
	Spruce	508 (45) ^{b}	4 (1) ^{b}	512 (45) ^{b}	235 (26) ^{a}	-1 (0) ^{a}	234 (27) ^{a}
	Birch	76 (23) ^{c}	0 (0) ^{a}	76 (23) ^{c}	235 (16) ^{a}	0 (0) ^{a}	235 (16) ^{a}
	No seedling	834 (19) ^{d}	10 (2) ^{c}	844 (20) ^{d}	193 (8) ^{a}	-1 (1) ^{a}	192 (9) ^{a}
Mineral soil	Pine	64 (19) ^{a}	1 (1) ^{a}	64 (18) ^{a}	-5 (18) ^{a}	0 (1) ^{a}	-4 (18) ^{a}
	Spruce	55 (9) ^{a}	1 (0) ^{a}	55 (8) ^{a}	129 (18) ^{b}	2 (1) ^{a}	131 (18) ^{b}
	Birch	58 (17) ^{a}	0 (0) ^{a}	59 (17) ^{a}	11 (11) ^{a}	0 (1) ^{a}	11 (11) ^{a}
	No seedling	237 (50) ^{b}	29 (4) ^{b}	266 (50) ^{b}	-143 (12) ^{c}	168 (29) ^{b}	26 (21) ^{a}

o.m. = organic matter

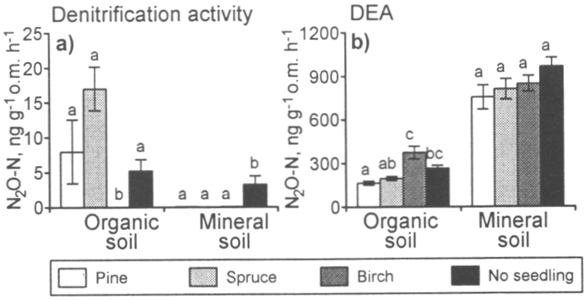


Fig. 3. a) Denitrification and b) denitrification enzyme activity of the soils. Values are means of five pots, bars show standard errors of the means. Values with the same letter are not significantly ($p \leq 0.05$) different from each other.

PLFA profiles

In the organic soil, the total amount of microbial lipids did not differ significantly between treatments, but the ratio of fungal to bacterial PLFAs was significantly higher in the birch rhizosphere than in the other soils (Table 3). PLFA profiles from the rhizosphere soils of all three tree species and the unplanted soil were distinct (Fig. 4a). Birch rhizosphere was most clearly separated from the unplanted soil along PC axis 1, which explained 42% of the variation. There were more of the fungal specific fatty acid 18:2ω6,9 and more branched fatty acids i16:1, i16:0, 10Me16:0, br17:0, br18:0 and 10Me17:0 in the birch rhizosphere (Fig. 5). PLFAs i17:0, 20:4 and C17:0 were relatively most abundant in the pine rhizosphere compared to the other samples. The amounts of 16:1ω7c and 18:1ω7 were also high in the pine rhizosphere compared to birch. The PLFAs common in the spruce rhizosphere, a15:0, 16:1ω5, 16:1ω7c and 18:1ω7, were similar to those abundant in the unplanted soil. The ratio of *trans* unsaturated 16:1ω7 to *cis* unsaturated 16:1ω7 was significantly higher in the birch rhizosphere (Table 3).

Table 3. The total amount of microbial PLFAs, ratio of fungal to bacterial PLFAs, and ratio of trans unsaturated 16:1ω7 to cis unsaturated 16:1ω7. Values are means of five pots, standard errors of the means in parentheses. Values with the same letter within one soil are not significantly ($p \leq 0.05$) different from each other.

Soil	Tree species	Microbial PLFAs, µmol g^{-1} o.m.	18:2ω6,9 : bacterial PLFAs	16:1ω7t : 16:1ω7c
Organic soil	Pine	1.4 (0.2) a	0.15 (0.01) a	0.29 (0.02) a
	Spruce	1.4 (0.1) a	0.14 (0.02) a	0.23 (0.01) ac
	Birch	1.7 (1.1) a	0.29 (0.01) b	0.43 (0.03) b
	No seedling	1.8 (0.1) a	0.12 (0.01) a	0.20 (0.01) c
Mineral soil	Pine	1.7 (0.2) a	0.11 (0.01) ab	0.17 (0.01) a
	Spruce	1.8 (0.2) a	0.17 (0.02) a	0.17 (0.02) a
	Birch	1.9 (0.2) a	0.18 (0.03) a	0.20 (0.02) a
	No seedling	1.7 (0.2) a	0.07 (0.02) b	0.15 (0.00) a

o.m. = organic matter

In the mineral soil, the total amount of microbial lipids did not differ between different treatments, but the ratio of fungal : bacterial PLFAs was significantly lower in the unplanted soil compared to spruce and birch rhizosphere soil (Table 3). Tree species did not clearly discriminate from each other in the PCA (Fig. 4b).

CLPPs

In the organic soil, the CLPPs separated birch rhizosphere from the other soils (Fig 6). All 125 C sources from GN and MT plates and 61 root exudate C sources did not differ in their separation power. The AWCD in the birch rhizosphere was considerably lower than in the other soils (results not shown). The top twenty C sources influencing the separation of birch from the other soils are shown in Table 4. The use of many phenolic acids was characteristic to birch rhizosphere. When using the C sources only from the MT plate, microbial communities from pine and spruce rhizosphere also had a tendency of separating from the unplanted soil (Fig. 6c). The substrates on MT plates had overall lower utilization rates than the ones on GN plates. In the mineral soil, CLPP from different tree species did not separate from each other in PCA with any substrate combinations (Fig. 7).

In some samples, there were significant, but not consistent, differences in the blank control wells and glucose containing wells in the GN and MT plates (results not shown).

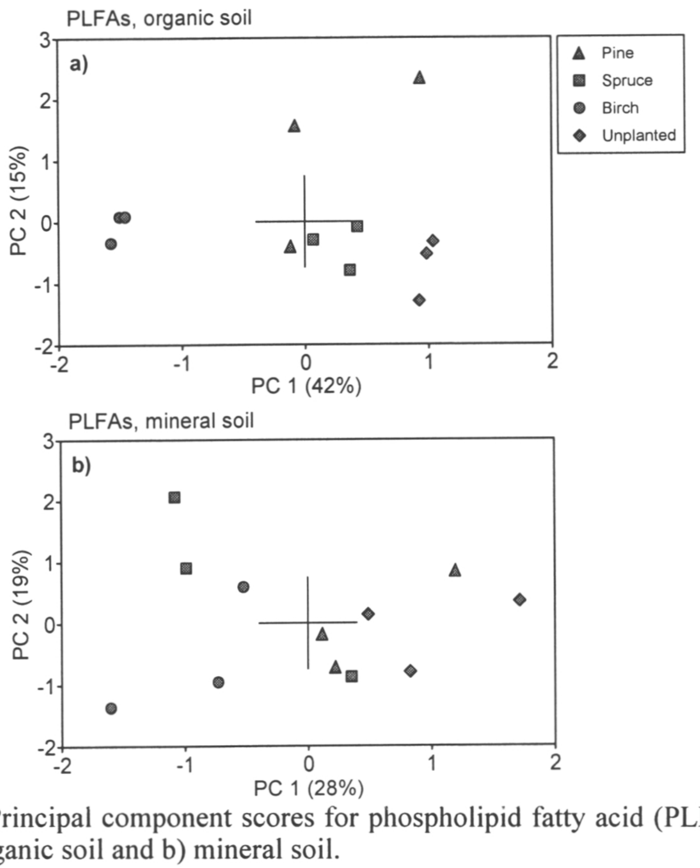


Fig. 4. Principal component scores for phospholipid fatty acid (PLFA) data on a) organic soil and b) mineral soil.

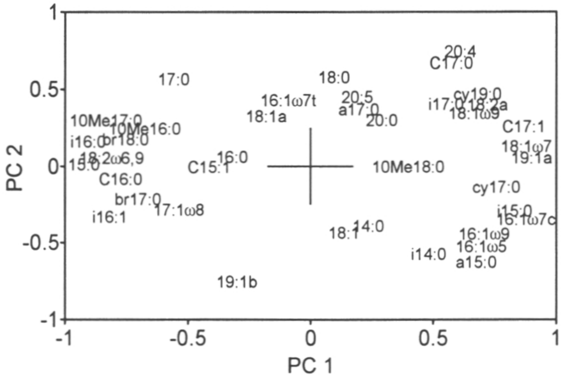


Fig. 5. Loading values for the individual PLFAs from the principal component analysis of the organic soils.

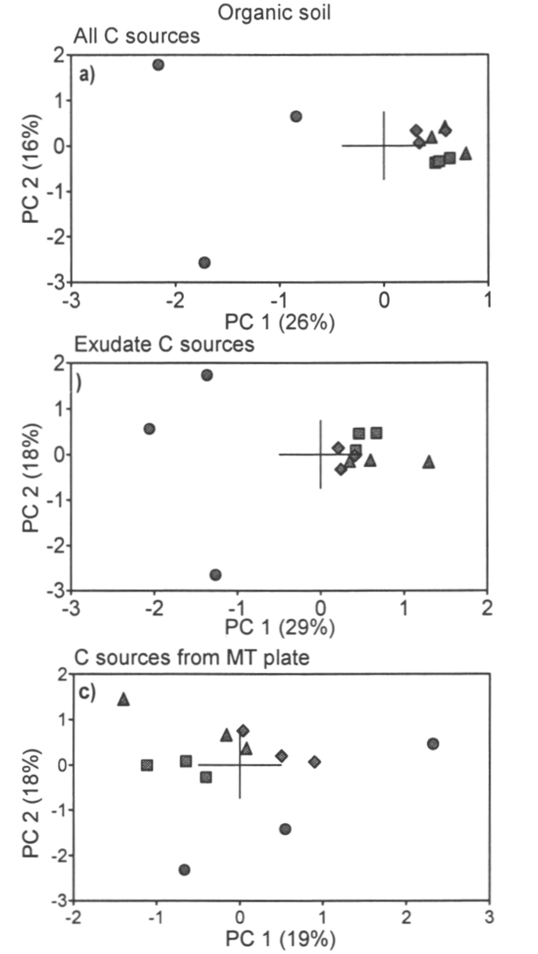


Fig. 6. Principal component scores for community level physiological profiles (CLPPs) in organic soils done with a) all 125 C sources, b) 61 root exudate C sources, and c) 31 C sources from MT-plates.

Table 4. List of top 20 C sources influencing most the separation of birch rhizosphere along PC axis 1. * indicates that the compound is from MT-plate.

Chemical group	All C sources	Loading for PC 1	Communities with greater utilization	Chemical group	Exudate C sources	Loading for PC 1	Communities with greater utilization
Carbohydrates	D-arabitol	0.91	Other soils	Carboxylic acids	levulonic acid*	-0.84	Birch
	N-acetyl-D-glucosamine	0.85	Other soils		citric acid	0.80	Other soils
					D,L-lactic acid	0.76	Other soils
Carboxylic acids	β-hydroxybutyric acid	0.93	Other soils		propionic acid	0.72	Other soils
	levulonic acid*	-0.88	Birch		oxaloacetic acid*	-0.71	Birch
	D-glucosaminic acid	0.82	Other soils		D-gluconic acid	0.71	Other soils
	mono-methyl succinate	-0.81	Birch		*cis* -aconitic acid	0.65	Other soils
	p -hydroxy phenylacetic acid	0.78	Other soils				
	citric acid	0.75	Other soils	Amino acids	L-aspartic acid	0.91	Other soils
	D,L-lactic acid	0.75	Other soils		L-histidine	0.86	Other soils
	fumaric acid*	-0.73	Birch		γ-amino butyric acid	0.85	Other soils
					L-glutamic acid	0.80	Other soils
Amino acids	γ-amino butyric acid	0.86	Other soils		L-phenylalanine	0.72	Other soils
	L-glutamic acid	0.81	Other soils		L-alanine	0.70	Other soils
	L-histidine	0.81	Other soils		hydroxy L-proline	0.70	Other soils
	L-aspartic acid	0.80	Other soils		glycine*	-0.64	Birch
Long chain aliphatic acids	oleic acid*	-0.92	Birch	Long chain aliphatic acids	oleic acid*	-0.96	Birch
Phenolic acids	ferulic acid*	-0.84	Birch	Phenolic acids	ferulic acid*	-0.81	Birch
	vanillic acid*	-0.83	Birch		vanillic acic*	-0.76	Birch
	chlorogenic acid*	-0.73	Birch		chlorogenic acid*	-0.71	Birch
					coumaric acid*	-0.70	Birch
Miscallaneous	inosine	-0.93	Birch				
	glycerol	0.90	Other soils				

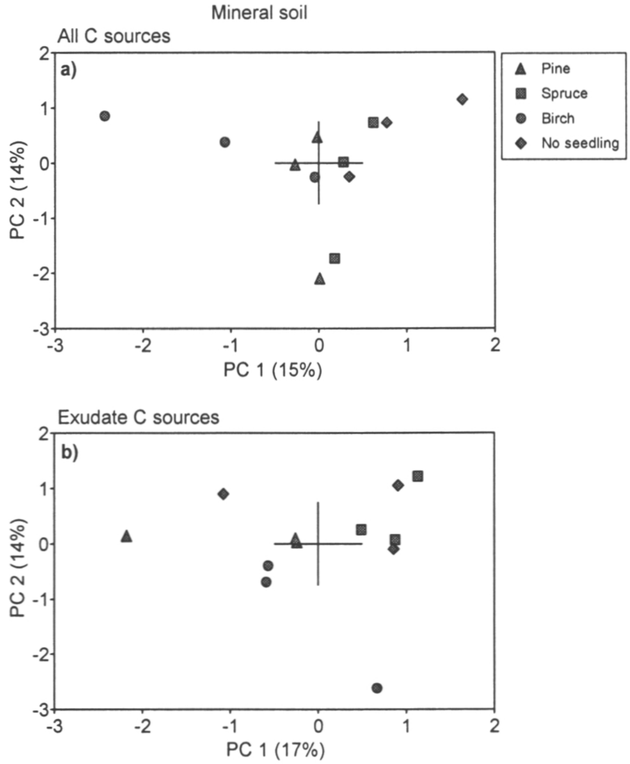


Fig. 7. Principal component scores for CLPPs in mineral soils done with a) all 125 C sources and b) root exudate C sources.

Plate counts

In the organic soil, numbers of colony-forming bacteria were lower in birch rhizosphere than in spruce rhizosphere (Table 5). There were negligible numbers of colony-forming pseudomonads in birch rhizosphere. Numbers of colony-forming fungi and yeasts did not differ between treatments.

In the mineral soil, numbers of colony-forming bacteria were significantly lower in unplanted soil, compared to all rhizosphere soils, and significantly lower in birch rhizosphere than in pine and spruce rhizosphere (Table 5). Numbers of colony-forming pseudomonads, fungi, and yeasts did not significantly differ between tree species.

Table 5. Results from plate counts of the soils. Values are means of three pots, standard errors of the means in parentheses. Values with the same letter within one soil are not significantly ($p \leq 0.05$) different from each other.

Soil	Tree species	Bacteria, cfu g^{-1} o.m. soil	Pseudomonads, cfu g^{-1} o.m. soil	Fungi cfu g^{-1} o.m. soil	Yeasts cfu g^{-1} o.m. soil
Organic soil	Pine	1.3×10^8 $(1.1 \times 10^7)^{ab}$	9.9×10^5 $(4.6 \times 10^5)^{a}$	1.5×10^6 $(2.4 \times 10^4)^{a}$	$< 10^{3}$ ^{a}
	Spruce	2.3×10^8 $(7.3 \times 10^7)^{a}$	9.1×10^5 $(2.7 \times 10^5)^{a}$	1.5×10^6 $(1.3 \times 10^5)^{a}$	2.9×10^5 $(2.7 \times 10^5)^{a}$
	Birch	4.2×10^7 $(3.4 \times 10^7)^{b}$	$< 10^{3}$ ^{c}	8.9×10^5 $(2.3 \times 10^5)^{a}$	1.9×10^5 $(1.1 \times 10^5)^{a}$
	No seedling	6.7×10^7 $(1.5 \times 10^7)^{ab}$	2.0×10^5 $(4.3 \times 10^4)^{b}$	9.3×10^5 $(2.6 \times 10^5)^{a}$	7.5×10^4 $(2.0 \times 10^4)^{a}$
Mineral soil	Pine	7.9×10^7 $(1.1 \times 10^7)^{a}$	3.3×10^6 $(7.8 \times 10^5)^{a}$	2.5×10^6 $(4.3 \times 10^5)^{a}$	1.1×10^6 $(4.1 \times 10^5)^{a}$
	Spruce	5.8×10^7 $(1.5 \times 10^7)^{a}$	1.4×10^6 $(6.8 \times 10^5)^{a}$	1.9×10^6 $(3.1 \times 10^4)^{a}$	8.5×10^5 $(2.4 \times 10^5)^{a}$
	Birch	2.1×10^7 $(6.4 \times 10^6)^{b}$	3.4×10^6 $(7.0 \times 10^5)^{a}$	2.2×10^6 $(3.0 \times 10^5)^{a}$	2.6×10^5 $(1.9 \times 10^5)^{a}$
	No seedling	6.1×10^6 $(8.5 \times 10^5)^{c}$	1.9×10^6 $(3.6 \times 10^5)^{a}$	2.7×10^6 $(2.4 \times 10^5)^{a}$	1.2×10^5 $(5.8 \times 10^4)^{a}$

cfu = colony forming units
o.m. = organic matter

Discussion

In field studies, birch species have often been found to raise soil pH, enhance the cycling of nutrients and stimulate microbial activities, whereas spruce species may do the opposite [22,23,24,11]. These effects are probably partly due to birch leaf litter containing more easily decomposable compounds than spruce needles [25,26]. However, the beneficial effect of birch may also arise from its root activities, a high rate of rhizodeposition [8,9]. Whether it is only that birch grows faster and has more roots and root tips releasing more exudates to soil microbes, or whether there are also qualitative differences in rhizodeposition, is not known.

In this study, where approximately similar volumes of soil on the roots of pine, spruce and birch seedlings were studied, some microbial characteristics differed between tree species, and some not. In the organic soil, C_{mic} and N_{mic} were significantly higher in birch rhizosphere than in pine and spruce rhizospheres, but SIR and basal respiration were not significantly different between the rhizosphere soils (Fig. 1). In the mineral soil, neither C_{mic}, N_{mic}, SIR or the basal respiration differed significantly in the rhizospheres of pine, spruce and birch.

SIR and basal respiration were significantly lower in unplanted mineral soil than in the rhizospheres of all tree species. This was probably because seedlings had provided soil microbes with an extra input of substrate, which had stimulated their growth, as was seen also by the significantly higher numbers of culturable bacteria in the rhizosphere soils than in the unplanted soil (Table 5). This is in agreement with the results of Parmelee et al [3], who found that in the organic soil pine roots and microbes competed with each other for moisture and N, but in nutrient-poor mineral soil roots provided the main input of substrate, which was more significant than the adverse effect of roots.

The concentration of mineral N was highest in both organic and mineral unplanted soil (Table 2), probably because of the absence of plant N uptake. In the organic soil, the concentration of mineral N was lowest in birch rhizosphere and highest in spruce rhizosphere. Correspondingly, N_{mic} was highest in birch rhizosphere and amount of N in needles/leaves of pine and birch higher than in those of spruce (Table 1, Fig. 1). In the mineral soil the amount of N in the seedlings or microbial biomass did not differ between different tree species, and neither did the concentration of mineral N in soil.

The net formation of mineral N did not differ between different tree species in the organic soil, but in the mineral soil it was highest in spruce rhizosphere. In other studies roots of trees have been shown both to increase and decrease N mineralization in soil [3,9]. Nevertheless, the higher net rate of mineral N formation in spruce rhizosphere could be due to higher microbial N uptake during the incubation in the other soils. Net nitrification was evident only in unplanted mineral soil (Table 3). This may be due to the fact that autotrophic nitrifiers are poor competitors with heterotrophic microbes. Therefore, in the organic soil and in the rhizosphere soils they have been outcompeted, but in the plantless mineral soil, where there are less heterotrophic microbes, they are active. This correlates with the fact that the numbers of ammonium and nitrite oxidizers were also slightly reduced in the mineral rhizosphere soils compared to the unplanted soil (Fig. 2).

Denitrification, as a process, is dependent on available C and low partial pressure of O_2, which is why denitrification is often enhanced in the rhizosphere [5,6]. However, in our study denitrification was only stimulated in spruce rhizosphere in the organic soil. Availability of substrate seemed to be the main factor controlling denitrification in these soils, because patterns of denitrification acticity followed the concentration of nitrate especially in the organic soils (Fig. 4a, Table 2). The activity of pre-existing denitrifying enzymes in soil, however, did not differ substantially between different tree species, as shown by DEA (Fig. 4b). On an organic matter basis, there was a higher DEA in the mineral soil compared to the organic soil, which could be due to lower partial pressure of O_2 in the compacting mineral soil, as compared to the more aerated organic soil. Denitrifying activity in the rhizosphere has been found to correlate with photosynthetic activity [27] and plant dry weight [28]. In our study there was a positive correlation between DEA and plant dry weight in the organic soils, but not in the mineral soils.

There were shifts in the soil microbial community structure in response to different tree species in the organic soil, but not in the mineral soil (Figs 4, 6, 7). One reason for this could be that in the organic soil there was more diversity to start with, which makes it possible that different groups are enriched in different conditions, whereas the original microbial community in the mineral soil probably was less diverse. Very little is known about the microbial communities in the rhizospheres of trees, with the exception of mycorrhizal fungi [29]. It has to be borne in mind that it is not only the roots of the trees that affect the microbial communities in soil, but also different mycorrhizal species and their exudates have been found to change the soil bacterial communities [30,31]. We did not determine how many and what kind of mycorrhizal infections the seedlings had in this study, but it may have been that seedlings were more mycorrhizal in the organic soil, as it is known that the highest concentration of mycorrhizal propagules are in the humus (organic) layer of soils [32].

The fatty acids more common in birch rhizosphere in the organic soil than in pine and spruce rhizosphere or in unplanted soil were the fungal specific 18:2ω6,9 (Fig. 5a), and branched fatty acids, which have commonly been found in Gram-positive bacteria [33]. The increasing amount of 18:2ω6,9 and the increased ratio of fungal to bacterial PLFAs from unplanted soil to birch rhizosphere was possibly due to higher populations of mycorrhizal fungi rather than saprophytes. The increase in the ratio of 16:1ω7t to 16:1ω7c in birch rhizosphere (Table 3) may indicate stress in the bacterial community, because an increase in the ratio of *trans*- to *cis*-monoenoic unsaturated PLFAs has been suggested to indicate starvation [34] or desiccation [35]. The latter explanation has been considered to be more probable [36]. It could be true also in our experiment, because despite the seedlings were watered to saturation every other day, the higher evapotranspiration from birch leaves caused the birch soils to be drier than the others during the hottest period of the summer.

The PLFA pattern of the pine rhizosphere in the organic soil separated slightly from the PLFA patterns of spruce and unplanted soil, but the changes in the individual PLFAs could not be clearly associated to certain groups of bacteria (Fig 5a). The PLFA patterns of the spruce rhizosphere and the unplanted soil were relatively similar. The PLFAs more common in those samples were mostly monounsaturated, typical to Gram-negative bacteria [37], even though the abundance of branched a15:0, common in Gram-positive bacteria, was also high. The relative amount of bacterial PLFA 16:1ω5 [38] was highest in the unplanted organic soil, and also higher in the spruce rhizosphere than under the other tree species. In the study of Frostegård et al. [39] 16:1ω5 decreased during incubation, and they suggested that this PLFA may reflect the dynamics of organisms that are responding to changes in the C status of soil. It could be that pine and birch have been taking up more nutrients from the soil than the smaller spruce seedlings, which has caused more competition between microbes and the plants and also less favourable C status of the soil towards the end of the growing season.

The CLPPs clearly differentiated only birch rhizosphere from the other soils (Figs 6, 7). There were negligible amounts of colony forming pseudomonads in the birch rhizosphere in the organic soil (Table 5), and the AWCD in Biolog plates from organic birch rhizospheres was very low, in spite of the bacterial inoculum densities being approximately the same as with other soils. This could influence the strong discrimination of birch by CLPPs, as the numbers of pseudomonads have been found to be directly correlated with colour development in Biolog wells [40]. The low number of *Pseudomonas* species in birch rhizosphere was surprising, given that this species is particularly stimulated in the rhizosphere (reviewed by Bolton et al. [41]). Nevertheless, fluorescent pseudomonads were commonly isolated from Scots pine mycorrhizospheres in nursery peat, but they were almost absent from outer mycorrhizospheres in pine forest humus, where *Bacillus* species were more important [30]. Because birch roots filled the pots almost totally, birch rhizosphere samples probably contained also soil around external hyphae of mycorrhizas, whereas pine and spruce rhizosphere samples included only soil around the roots. Thus, there might have been a higher dominance of *Bacillus* species in birch rhizosphere samples compared to those of pine and spruce, as indicated also by the PLFA profiles, which showed a high amount of Gram-positive bacteria in birch rhizosphere.

There has been a lot of discussion regarding the reliability of methods for measuring microbial community structure and function and indeed what they do measure. PLFAs are located in the cell membranes, and due to the presence of specific "signature" lipids in different microorganisms are therefore a measure of the structure of the community [42]. Microbes may, however, change the lipid content of their membranes in changing conditions. Therefore changes in PLFA patterns can also partly reflect changes within one species. However, there are no studies which have shown that such energy-demanding alterations would happen in nutrient-poor soil environment [42], while the shift in humus bacterial PLFA composition has been shown to be connected to changes in the species composition of the culturable bacteria in humus [43]. The determination of CLPPs was suggested as a method to measure the functional diversity of microbial communities. It must be remembered, however, that CLPPs measure potential, rather than actual substrate use of the community, and CLPPs may therefore be better considered as measuring the structural rather than functional diversity of the community [44,45]. Many studies have found that there are changes in the PLFA patterns, but no changes in CLPP patterns, or the changes are less clear and the variation higher [43,45,46]. This can either mean that the microbial communities change their structure without changing their functions, or that the Biolog method is less sensitive in detecting changes in the microbial community structure than PLFAs. This latter case is probably true, due to the fact that PLFAs assess the whole community, whereas CLPPs only measure the metabolic profiles of the culturable bacteria [18]. The differences in glucose containing wells in GN and MT plates found in our study probably indicated the large microscale heterogeneity in the composition of soil microbial communities. Haack et al. [47] found that although whole-community substrate utilization profiles were reproducible for a given community, replicate soil samples varied greatly in the rate and extent of oxidation of single substrates.

It is plausible to think that using ecologically relevant C sources, such that can be found from soils, would give the best separation with CLPPs. Campbell et al. [17] found the separation of microbial communities to be more distinct using the 61 exudate C sources than the 125 C sources in GN and MT plates together. In this study, however, this was not the case (Fig. 6). The C sources in the MT plate alone, however, had a tendency of separating also pine and spruce rhizosphere from the unplanted soil. This might indicate that the substrates in MT plates were more ecologically relevant for these samples than the ones in GN plates.

In conclusion, in the organic soil C_{mic} and N_{mic} were higher in birch rhizosphere than in pine and spruce rhizosphere. Also the microbial community structure in the rhizospheres of pine, spruce and birch and in the unplanted soil were distinct. In spite of this, rates of C and N mineralization were not different between treatments. In the mineral soil all tree roots stimulated C mineralization in soil, and prevented nitrification. In addition, the size of the microbial biomass, and microbial community structure did not differ in the rhizospheres of different tree species. Thus, in the mineral soil the strongest effect on soil microbes was the presence of a plant, but in the organic soil different tree species had a significant influence on soil microbes.

Acknowledgements
We thank the staff at Ruotsinkylä field station for taking care of the plants. Anneli Rautiainen, Terhi Hallantie, Eileen J. Reid and Ruth MacDougall are thanked for help in the laboratory. We are grateful for the Academy of Finland and the foundations Metsämiesten säätiö and Emil Aaltosen säätiö for supporting this work financially.

References

[1] Wardle, D.A. (1992) A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol. Rev. 67, 321-358.

[2] Bååth, E., Lohm, U., Lundgren, B., Rosswall, T., Söderström, B., Sohlenius, B. and Wirén, A. (1978) The effect of nitrogen and carbon supply on the development of soil organism populations and pine seedlings: a microcosm experiment. Oikos 31, 153-163.

[3] Parmelee, R.W., Ehrenfeld, J.G. and Tate III, R.L. (1993) Effects of pine roots on microorganisms, fauna, and nitrogen availability in two soil horizons of a coniferous forest spodosol. Biol. Fertil. Soils 15, 113-119.

[4] Norton, J.M. and Firestone, M.K. (1996) N dynamics in the rhizosphere of *Pinus ponderosa* seedlings. Soil Biol. Biochem. 28, 351-362.

[5] Wollersheim, R., Trolldenier, G. and Beringer, H. (1987) Effect of bulk density and soil water tension on denitrification in the rhizosphere of spring wheat (*Triticum vulgare*). Biol. Fertil. Soils 5, 181-187.

[6] Wheatley, R., Ritz, K. and Griffiths, B. (1990) Microbial biomass and mineral N transformations in soil planted with barley, ryegrass, pea or turnip. Plant and Soil 127, 157-167.

[7] Grayston, S.J and Campbell, C.D. (1996) Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (*Larix eurolepis*) and Sitka spruce (*Picea sitchensis*). Tree Physiol. 16, 1031-1038.

[8] Priha, O., Lehto, T. and Smolander, A. (1998) Mycorrhizas and C and N transformations in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings. Plant and Soil 206, 191-204.

[9] Bradley, R.L. and Fyles, J.W. (1995) Growth of paper birch (*Betula papyrifera*) seedlings increases soil available C and microbial acquisition of soil-nutrients. Soil Biol. Biochem. 27, 1565-1571.

[10] Cajander A. K. (1949) Forest types and their significance. Acta For. Fenn. 56, 1-71.

[11] Priha, O. and Smolander, A. (1999) Nitrogen transformations in soil under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. Soil Biol. Biochem. 31, 965-977 (in press).

[12] Agerer, R. (Ed.) (1987-96) Colour Atlas of Ectomycorrhizae. Einhorn-Verlag Eduard Dietenberger, Schwäbisch Gmünd, D-7070 Germany.

[13] Priha, O. and Smolander, A. (1997) Microbial biomass and activity in soil and litter under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at originally similar field afforestation sites. Biol. Fertil. Soils 24, 45-51.

[14] Luo, J., White, R.E., Ball, P.R. and Tillman, R.W. (1996) Measuring denitrification activity in soils under pasture: optimizing conditions for the short-term denitrification enzyme assay and effect of soil storage on denitrification. Soil Biol. Biochem. 28, 409-417.

[15] Frostegård, Å., Bååth, E. and Tunlid, A. (1993) Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol. Biochem. 25, 723-730.

[16] Frostegård, Å. and Bååth, E. (1993) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils 22, 59-65.

[17] Campbell, C.D., Grayston, S.J. and Hirst, D.J. (1997) Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. J. Microb. Meth. 30, 33-41.

[18] Garland, J.L. and Mills, A.L. (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57, 2351-2359.

[19] Garland, J.L. (1996) Analytical approaches to the characterisation of samples of microbial communities using patterns of potential source utilisation. Soil Biol. Biochem. 28, 213-221.

[20] Ranta, E., Rita, H., and Kouki, J. (1989) Biometria, 2nd Edn., 569 pp. Yliopistopaino, Helsinki.

[21] Mustonen, S. (1995) Tilastolliset monimuuttujamenetelmät. 205 pp. Yliopistopaino, Helsinki.

[22] Miles, J. and Young, W.F. (1980) The effects on heathland and moorland soils in Scotland and northern England following colonization by birch (*Betula* spp.). Bull. Ecol. (France) 11, 233-242.

[23] Mikola, P. (1985) The effect of tree species on the biological properties of forest soil. Nat. Swed. Env. Prot. Board 3017, 27 p.

[24] Ranger, J. and Nys, C. (1994) The effect of spruce (*Picea abies* Karst.) on soil development: an analytical and experimental approach. Eur. J. Soil Sci. 45, 193-204.

[25] Nykvist, N. (1963) Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter. Stud. For. Suec. 3, 1-31.

[26] Johansson, M.-B. (1995) The chemical composition of needle and leaf litter from Scots pine, Norway spruce and white birch in Scandinavian forests. Forestry 68, 49-62.

[27] Scaglia, J., Lensi, R. and Chalamet, A. (1985) Relationship between photosynthesis and denitrification in planted soil. Plant and Soil 84, 37-43.

[28] Hall, J.M., Paterson, E. and Killham, K. (1998) The effect of elevated CO_2 concentration and soil pH on the relationship between plant growth and rhizosphere denitrification potential. Global Change Biol. 4, 209-216.

[29] Grayston, S.J., Vaughan, D. and Jones, D. (1996) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl. Soil Ecol. 5, 29-56.

[30] Timonen, S., Jørgensen, K.S., Haahtela, K. and Sen R. (1998) Bacterial community structure at defined locations of *Pinus sylvestris-Suillus bovinus* and *-Paxillus involutus* mycorrhizospheres in dry pine forest humus and nursery peat. Can. J. Microbiol. 44, 499-513.

[31] Olsson, P.A. (1998) The external mycorrhizal mycelium. PhD thesis, Department of Ecology, Lund University, Sweden.

[32] Entry, J.A., Rose, C. L. and Cromack, K. (1991) Litter decomposition and nutrient release in ectomycorrhizal mat soils of a Douglas fir ecosystem. Soil Biol. Biochem. 23, 285-290.

[33] O'Leary, W.M. and Wilkinson, S.G. (1988) Gram-positive bacteria. In: Microbial Lipids, Vol 1. (Ratledge, C. and Wilkinson, S.G., Eds.) pp. 117-185. Academic Press Ltd., London.

[34] Guckert, J.B., Hood, M.A. and White, D.C. (1986) Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increases in the *trans/cis* ratio and proportions of cyclopropyl fatty acids. Appl. Environ. Microbiol. 52, 794-801.

[35] Kieft, T.L., Ringelberg, D.B. and White D.C. (1994) Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Appl. Environ. Microbiol. 60, 3292-3299.

[36] Keweloh, H. and Heipieper, H.J. (1996) *Trans* unsaturated fatty acids in bacteria. Lipids 31, 129-137.

[37] Wilkinson S.G. (1988) Gram-negative bacteria. In: Microbial Lipids, Vol 1. (Ratledge, C. and Wilkinson, S.G., Eds.) pp. 299-488. Academic Press Ltd., London.

[38] Nichols, P.D., Stulp, B.K., Jones, J.G. and White, D.C. (1986) Comparison of fatty acid content and DNA homology of the filamentous gliding bacteria *Vitreoscilla*, *Flexibacter*, and *Filibacter*. Arch. Microbiol. 146, 1-6.

[39] Frostegård, Å., Tunlid, A. and Bååth, E. (1996) Changes in microbial community structure during long-term incubation in two soils experimentally contaminated with metals. Soil Biol. Biochem. 28, 55-63.

[40] Grayston, S.J., Wang, S., Campbell, C.D. and Edwards, A.C. (1998) Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol. Biochem. 30, 369-378.

[41] Bolton, H., Fredrickson, J.K. and Elliott, L.F. (1992) Microbial ecology of the rhizosphere. In: Soil Microbial Ecology (Metting, F.B., Ed.) pp. 27-63. Marcel Dekker, New York.

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

[43] Pennanen, T., Fritze, H., Vanhala, P., Kiikkilä, O., Neuvonen, S. and Bååth E. (1998) Structure of a microbial community in soil after prolonged addition of low levels of simulated acid rain. Appl. Environ. Microbiol. 64, 2173-2180.

[44] Garland, J.L., Cook, K.L., Loader, C.A. and Hungate, B.A. (1997) The influence of microbial community structure and function on community-level physiological profiles. In: Microbial Communities, Functional Versus Structural Approaches (Insam, E. and Ranger, A., Eds.) pp. 171-183. Springer-Verlag, Berlin, Heidelberg.

[45] Bååth, E., Díaz-Raviña, M., Frostegård, Å. and Campbell, C.D. (1998) Effect of metal-rich sludge amendments on the soil microbial community. Appl. Environ. Microbiol. 64, 238-245.

[46] Buyer, J.S. and Drinkwater, L.E. (1997) Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. J. Microb. Meth. 30, 3-11.

[47] Haack, S.K., Garchow, H., Klug, M.J. and Forney, L.J. (1995) Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Appl. Environ. Microbiol. 61, 1458-1468.

ISBN 951-40-1678-5
ISSN 0358-4283
Hakapaino 1999