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

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Preface

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# Contents

List of original publications

1. **Introduction** ................................................................. 7  
   1.1 Mechanisms by which trees affect soils ...................... 7  
   1.2 Birch versus conifers ................................................ 8  
   1.3 Rhizosphere as a habitat for microbes .................... 11  
   1.4 Studying soil microbes ............................................. 14  

2. **The aim of the study** .................................................... 17  

3. **Materials and methods** ................................................ 17  
   3.1 Field sites ............................................................... 17  
   3.2 Greenhouse experiments .......................................... 18  
   3.3 Microbial determinations ......................................... 18  
   3.4 Soil physical and chemical analyses ......................... 19  
   3.5 Analyses of the seedlings ....................................... 20  
   3.6 Statistical analyses ................................................. 20  

4. **Results and discussion** ............................................... 21  
   4.1 Chemical properties of soils under Scots pine, Norway  
       spruce and silver birch .......................................... 21  
   4.2 Soil microbial biomass and C mineralization rate in  
       stands of different tree species ............................ 22  
   4.3 Response of soil N transformations to tree species .... 24  
   4.4 Microbial biomass and C mineralization rate in  
       the rhizospheres .................................................. 27  
   4.5 N transformations in the rhizospheres ...................... 29  
   4.6 The influence of tree species on microbial communities... 33  
   4.7 Concluding remarks ............................................... 37  

5. **Summary** ...................................................................... 38  

References ........................................................................... 39
Original publications

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


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.


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 watersoluble 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 N2-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, Sætre 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 metabolic 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 non-mycorrhizal 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, algae, 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 CO₂ 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 18:2o6,9 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 N₂O production in the presence of acetylene, which blocks nitrous oxide reductase, thus causing the sole end product of denitrification to be N₂O (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 N₂O 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 N₂-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 ¹⁵N (Myrold and Tiedje 1986). ¹⁵N can be used as a tracer, which reveals the relative rates and partitioning of added ¹⁵N, 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 Urainen 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 Karrikkala 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$_2$O-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 CaCl₂ (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(H₂O) 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 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(H₂O) 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).

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 18:2ω6,9 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 m³,
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 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
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$_4$)$_2$SO$_4$ 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 O₂ 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 16:1ω7c to 16:1ω7t 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 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.

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