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Responses of soil microbial communities to clonal
variation of Norway spruce

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Academic dissertation

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ABSTRACT

In boreal forests, microorganisms have a pivotal role in nutrient and water supply of trees as well as in litter decomposition and nutrient cycling. This reinforces the link between above-ground and below-ground communities in the context of sustainable productivity of forest ecosystems. In northern boreal forests, the diversity of microbes associated with the trees is high compared to the number of distinct tree species. In this thesis, the aim was to study whether conspecific tree individuals harbour different soil microbes and whether the growth of the trees and the community structure of the associated microbes are connected.

The study was performed in a clonal field trial of Norway spruce, which was established in a randomized block design in a clear-cut area. Since out-planting in 1994, the spruce clones showed two-fold growth differences. The fast-growing spruce clones were associated with a more diverse community of ectomycorrhizal fungi than the slow-growing spruce clones. These growth performance groups also differed with respect to other aspects of the associated soil microorganisms: the species composition of ectomycorrhizal fungi, in the amount of extraradical fungal mycelium, in the structure of bacterial community associated with the mycelium, and in the structure of microbial community in the organic layer. The communities of fungi colonizing needle litter of the spruce clones in the field did not differ and the loss of litter mass after two-years decomposition was equal. In vitro, needles of the slow-growing spruce clones were colonized by a more diverse community of endophytic fungi that were shown to be significant needle decomposers.

This study showed a relationship between the growth of Norway spruce clones and the community structure of the associated soil microbes. Spatial heterogeneity in soil microbial community was connected with intraspecific variation of trees. The latter may therefore influence soil biodiversity in monospecific forests.

Keywords: Diversity, growth, ectomycorrhiza, endophytic fungi, litter decomposition, *Picea abies* (L.) Karst

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Espoo, February 2008



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AUTHOR'S CONTRIBUTION

- I Tiina Rajala wrote the paper, planned the experiment together with co-authors and performed the experimental work except the microsatellite analysis. She interpreted the results together with co-authors.
- II Tiina Rajala wrote the paper, participated in planning of the study and carried out the experimental work except the PLFA analysis. She interpreted the results.
- III Tiina Rajala wrote the paper, contributed to designing of the study and performed part of the experimental work. She interpreted the results.
- IV Tiina Rajala wrote the paper, contributed to designing of the study and carried out the experimental work except the analysis of understorey vegetation, the canonical discriminant analysis and the non-metric multidimensional scaling. She interpreted the results together with co-authors.

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ABBREVIATIONS

cDNA	Complementary deoxyribonucleic acid
DGGE	Denaturing gradient gel electrophoresis
d.m.	Dry matter
ECM	Ectomycorrhiza
ITS	Internal transcribed spacer
o.m.	Organic matter
PCR	Polymerase chain reaction
PLFA	Phospholipid fatty acid
rDNA	Ribosomal deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction

1 INTRODUCTION

1.1 Interactions between above-ground and below-ground communities

The importance of biodiversity for the functioning of ecosystems has gained much interest during the last decades, as global climate change and other human-induced alterations of the environment are driving major species extinction (Chapin et al. 2000, McCann 2000). The relationship between community structure and ecosystem productivity on the one hand and species diversity and ecosystem stability on the other, is a widely studied but debated issue (Chapin et al. 1997, Waide et al. 1999, Loreau et al. 2001, Naeem 2002, Coleman and Whitman 2005). The investigation of the diversity-function relationship is beset by several problems (Bengtsson 1998, Wardle 1999), and it seems that patterns between species diversity and ecosystem functions are highly variable and dependent on context. Hitherto, studies of terrestrial ecosystems have mainly focused on above-ground ecosystems, ignoring the properties and responses of below-ground ecosystems. However, understanding the functioning of the whole ecosystem requires experiments that involve higher-level interactions, instead of concentrating on a single trophic level (McCann 2000).

In the boreal forest ecosystem, trees grow together with a diverse community of soil microorganisms, and associated microbes are the basis for the natural growth and performance of trees. Biotic interactions between trees and microbes vary from symbiotic to pathogenic and the net of direct and indirect feedbacks between trees and microbes can be highly complex (Bever 2003, Wardle et al. 2004). Two groups of microorganisms having a pivotal role in boreal forest soil ecosystems are ectomycorrhizal (ECM) and saprotrophic fungi. Symbiotic ECM fungi are essential for nutrient and water supply of trees, whereas saprotrophic microbes have a key role in the decomposition of organic residues and nutrient cycling. The interactions between above-ground and below-ground communities are the basis for the stability and function of the whole forest ecosystem. Influence of the above-ground species diversity or identity on the structure and activity of below-ground microbial community may have ecosystem-level consequences with impacts on forest productivity.

Trees change soil properties so strongly as to create patchiness in soil microbial communities (Pennanen et al. 1999, Saetre and Bååth 2000). Tree species differ in the quantity and quality of resources they provide for soil microbes (Grayston et al. 1996, Murphy et al. 1998). Moreover, specificity in mycorrhizal symbiosis varies, meaning that ECM composition may differ among host trees, particularly among tree genera or families (Molina et al. 1992). As a consequence, the tree species composition and diversity may affect activity and the structure of soil microbial communities (Priha 1999, Kernaghan et al. 2003, Wardle et al. 2004, Ishida et al. 2007, Kanerva 2007). In northern boreal forests, however, the diversity of tree species is very low compared to the soil microbial diversity. Despite the low richness of tree species, the intraspecific genetic variation within populations of conifers is high (Muona 1990).

One of the most common and commercially important coniferous species in Europe is Norway spruce. In Finland, Norway spruce has a major role in forestry; in 2005 it comprised 44% of commercial roundwood removal (Peltola 2006). Long-term breeding programmes, which are based on testing, selection and crossing of trees with desired characters, have improved productivity and quality of Norway spruce. However, factors and their heritability that affect vigour of the trees are poorly understood (Mari et al. 2003a). Spruce growth and wood quality are known to have a genetic basis (Rozenberg and Cahalan 1997, Mari et al. 2003b, Hannrup et al. 2004) and responses of spruce clones to various stress factors, such as

drought and ozone, vary (Karlsson et al. 1997, Sonesson and Eriksson 2003). Norway spruce clones are also known to differ in their resistance against soil pathogenic fungi (Swedjemark et al. 1998, Hietala et al. 2003, Karlsson and Swedjemark 2006). Yet, it remains to be explored whether the identity of Norway spruce has an effect on the mutualistic fungi and soil microbial community as a whole, and whether the vigour of Norway spruces is indirectly affected by their specific effect on the associated soil microbial communities.

1.2 Ectomycorrhizal communities

1.2.1 Definition and general background of ectomycorrhizas

The mycorrhizal symbiosis is a common association between plant roots and fungi; in nature the majority of terrestrial plant roots are colonized by symbiotic fungi forming mycorrhizas (Pirozynski and Malloch 1975, Malloch et al. 1980, Smith and Read 1997). Term “mycorrhiza” was coined by A.B. Frank already in 1885 (Frank 1885), but it took 50 years before some of his hypothesis were accepted and confirmed (Trappe 2005). In mycorrhizal symbiosis, the fungus captures nutrients effectively from the soil and translocates part of them to the host plant. In return, the host plant supports mycorrhizal fungi by delivering photosynthesized carbohydrates. Estimates of the quantity of assimilates allocated to the fungi varies widely; in ectomycorrhizal symbiosis it may be 10–50% (Smith and Read 1997, Simard et al. 2002). The fungi not only supply the host plant with nutrients but may also defend the host against drought, pathogens and heavy metals (Smith and Read 1997). In general, mycorrhizal symbiosis refers to a mutualistic association that benefits both partners. However, in some cases the association between plant and mycorrhizal fungus can be neutral or even turn antagonistic (Egger and Hibbett 2004).

Ectomycorrhizal symbiosis is a common mycorrhiza type that is characterized by intercellular fungal colonization. The other mycorrhizal associations are arbuscular mycorrhiza, ectendomycorrhiza, arbutoid mycorrhiza, monotropoid mycorrhiza, ericoid mycorrhiza and orchid mycorrhiza (Smith and Read 1997). Ectomycorrhizas are typical for short roots of woody plants, such as members of Pinaceae, Betulaceae, Fagaceae and Salicaceae and it is the prevalent mycorrhizal type in northern boreal and temperate forests. Ectomycorrhizal fungi are mainly basidiomycetes but ascomycetes and some zygomycetes also contain ECM fungal species (Molina et al. 1992).

An ECM root tip can be recognized from the labyrinth-like structure of the root cross-section. This structure, called a Hartig net, is formed as the ECM fungus grows between the plant cortical cells, and it is the part of the mycorrhiza where nutrient and carbon exchange occurs (Harley and Smith 1983). Usually, the ECM fungus also grows on the root surface. Multiple layers of fungal hyphae form a sheath, i.e., a mantle. Fungal hyphae extending from the surface of the mantle form extraradical mycelium. Morphological and anatomical features of ectomycorrhizas are more or less characteristic to fungal species. These characters include colour, occurrence and abundance of cystidia, emanating hyphae and rhizomorphs, shape and size of cells in mantle layers, shape and diameter of emanating hyphae, cystidia, clamp connections and rhizomorphs, and thickness of their cell walls (Agerer 1991, Tedersoo 2007).

Boreal forest soils are poor in easily accessible nutrients (Tamm 1991). The formation of ectomycorrhizas allows plants to be competitive against other soil organisms in order to obtain nutrients (Smith and Read 1997, Lindahl et al. 2002). Nearly all short roots of boreal forest

trees are colonized by ECM fungi (Taylor et al. 2000). Plants provided with sufficient amount of inorganic nutrients are able to grow without mycorrhizas but in the field, non-mycorrhizal seedlings do not survive as well as mycorrhizal seedlings (Marx et al. 1977, Wilson et al. 1987). Ectomycorrhizal fungi increase uptake of dissolved nutrients but can also render nutrients available from forms and complexes that are inaccessible for plants (Harley and Smith 1983, Rygielwicz et al. 1984, Lindahl et al. 2002). With extracellular enzymes, ECM fungi mobilize nutrients from organic sources and through organic acid excretion they capture nutrients from mineral sources (Chalot and Brun 1998, Wallander 2000, Landeweert et al. 2001). Enzyme activities of ECM fungal species differ (Courty et al. 2005) and accordingly, nutrient uptake rates and utilization of different complexes vary among ECM fungi (Abuzinadah and Read 1986, Abuzinadah and Read 1989, Dighton et al. 1990, Finlay et al. 1992, Wallander et al. 2003). ECM fungi not only improve the nutrient supply but also can transport water to the host tree and protect roots against drought, pathogens and heavy metals (Smith and Read 1997). It has long been noted that ECM fungi differ in their ability to enhance the growth of hosts (Theodorou and Bowen 1970). However, the results of the impact of different ECM fungi have been somewhat inconsistent (Mikola 1973). This may be accounted for by different ECM fungal strains, tree species, tree genotypes and experimental conditions.

Ectomycorrhizal symbiosis is evolutionary unstable and it has evolved independently among multiple lineages of fungi (Hibbett et al. 2000). Phylogenetic studies have revealed that, for example, agarics, which include the Boletaceae and Russulaceae, are derived from wood-rotting fungi (Moncalvo et al. 2000). The common ancestors among mycorrhizal and saprotrophic fungi may explain why ECM fungi express a range of saprotrophic capabilities. Many ECM fungi are able to degrade organic compounds and many ECM fungi inhabit decaying organic matter (Lindahl et al. 2002, Read and Perez-Moreno 2003). Hence, saprotrophic and mycorrhizal fungi are not completely distinct guilds, but their habitats and functions are partly overlapping (Read and Perez-Moreno 2003, Steffen 2003).

Ectomycorrhizal communities are tremendously diverse. Worldwide, 5000–6000 fungal species may be capable of forming ECM (Molina et al. 1992). Many edible mushrooms, such as highly prized *Tuber* sp., *Tricholoma matsutake* and *Boletus edulis*, as well as members of the genera *Russula*, *Lactarius* and *Cantharellus* are ectomycorrhizal. On the other hand, genera containing highly poisonous species, like *Amanita* and *Cortinarius*, form ECM associations as well. Relatively well known and widely explored are those ECM fungal species that form easily recognized epigeous fruiting bodies. However, since the 1990s the use of DNA methods for studies of ECM diversity has revealed a large proportion of the ECM fungi that have hitherto been overlooked, because they produce inconspicuous hypogeous or resupinate fruiting bodies (Horton and Bruns 2001). In boreal forests, for instance, species in genera *Tylospora*, *Amphinema*, *Piloderma*, *Tomentella* and *Tomentellopsis* are prominent (Kõljalg et al. 2000, Taylor et al. 2000, Tedersoo et al. 2003, Toljander et al. 2006). Although DNA analyses are useful for identification of ECM fungi, occasionally a fungus extracted from an environmental sample remains unidentified, because the obtained fingerprint or sequence does not match those of any previously described species. This indicates that still only some of the ECM fungal species have been described and species diversity is underestimated. Many non-described species are likely to be found from tropical and southern areas, which are less intensively studied than the northern forest. Moreover, many fungi, such as Tomentelloid fungi, were previously considered as saprotrophic but nowadays are categorized as ECM fungi (Kõljalg et al. 2000). Therefore, earlier estimates of ECM richness may be too low and a more accurate number of ECM fungal species may be near 10 000 (Taylor and Alexander 2005).

1.2.2 Causes and effects of ectomycorrhizal diversity

Numerous factors may affect the diversity of an ECM community. Formation of an ECM symbiosis in forest soil depends on host species, age and vigour of the trees, edaphic and environmental conditions, availability of fungal inoculum, competition, microflora and microfauna (Deacon and Fleming 1992). Anthropogenic stress, site history, habitat size and degree of isolation are also likely to affect the ECM community structure (Erland et al. 1999, Peay et al. 2007). Diversity of host trees can contribute to the ECM diversity (Kernaghan et al. 2003, Ishida et al. 2007). Specificity in mycorrhizal symbiosis may affect ECM community structure, since although many ECM fungi have a broad plant host range, some ECM fungal species are not shared among coexisting host trees. (Molina et al. 1992, Newton and Haigh 1998). The host tree may also affect the ECM community through the quality of litter (Conn and Dighton 2000) or through effects on soil chemical, physical and biological properties. Bruns (1995) hypothesized that in a monoculture forest stand, ECM diversity might result from resource partitioning, soil disturbance and competitive interactions between ECM fungal species. Yet, the causes of resource heterogeneity are largely unknown and the hypothesis that intraspecific variation of trees affects local ECM diversity is thus far neglected.

Ectomycorrhizal formation is known to be under genetic control. Intraspecific variation in the ability of host plants to form ECM symbiosis with a single fungal isolate has been documented (Walker et al. 1986, Tonkin et al. 1989, Rosado et al. 1994b, Tagu et al. 2001). However, the influence of intraspecific variation of host trees on the ECM community structure (i.e. species composition and diversity) is still unknown. Gehring et al. (1998) found that dominant ECM species differed among coexisting *Pinus edulis* individuals. In contrast, Saari et al. (2005) did not observe difference in ECM communities among *Pinus sylvestris* individuals. Although Saari et al. stated that the lack of difference may have been due to unequal sampling of each host individual.

The diversity of arbuscular mycorrhizal fungi is thought to have a positive effect on the productivity of grasses (van der Heijden et al. 1998, Maherali and Klimoros 2007). Similarly, in spatially heterogeneous forest soils, high ECM diversity may improve the growth of the host tree (Reddy and Natarajan 1997, Baxter and Dighton 2001, Nara et al. 2003). However, the effect of ECM diversity on tree productivity seems to be dependent on context, e.g. soil nutrient levels (Jonsson et al. 2001), and consensus on the importance of mycorrhizal diversity for the above-ground ecosystem is lacking. More obviously, the composition of the ECM community is essential for vigour of trees and species have to be well adapted to the soil conditions (Kranabetter 2004). As already discussed, the positive effect on the host plant may vary among different ECM fungal species. On the other hand, carbon demand (Colpaert et al. 1996) or stimulation of photosynthesis (Dosskey et al. 1990) of different ECM fungal species differs. Therefore, the cost-benefit ratio of ECM fungi to the host tree varies, emphasizing the importance of compatibility between the partners of the symbiosis. However, it has been suggested that some degree of functional redundancy exists in ECM communities and the activity of key species or functional groups is the most important for ecosystem productivity and function (Perry et al. 1989, Dahlberg 2001).

1.2.3 Investigation of ectomycorrhizal communities

It is well recognized that the ECM community differs from the above-ground and below-ground points of view (Dahlberg et al. 1997, Kåren and Nylund 1997, Gehring et al. 1998, Jonsson et al. 1999, Horton and Bruns 2001). The observed discrepancy between above-

ground and below-ground ECM fungal communities may partly be attributable to sampling differences (Horton and Bruns 2001). On the other hand, hidden or cryptic ECM fungal species are overlooked if ECM fungal communities are investigated only above-ground. Therefore, sporocarp sampling survey is considered an inadequate method for investigation of ECM community (Dahlberg 2001).

The dynamics of ECM communities make them challenging to sample. Temporal changes in ECM fungal communities are still poorly known, but there are some studies indicating temporal variation in ECM community structures (Kjøller 2006, Koide et al. 2007). Therefore, a single sampling event represents the ECM community only at a particular moment. ECM communities are also spatially heterogeneous and ECM fungi show variable distribution patterns (Tedersoo et al. 2003, Koide et al. 2005, Genney et al. 2006). Due to the patchiness, the number and size of collected soil samples may have an impact on the observed ECM community structure (Menkis et al. 2005). Commonly, the investigation of ECM communities is restricted to the organic soil layer. Although organic soil, along with the uppermost mineral layer, usually has the highest living fine root density and biomass (Makkonen and Helmisaari 1998, Rosling et al. 2003), mineral soil deeper in the ground also contains a lot of root tips. As ECM communities are found to be vertically distributed and some ECM fungal species are restricted to the mineral soil (Heinonsalo et al. 2001, Landeweert et al. 2001, Rosling et al. 2003, Tedersoo et al. 2003), sampling mycorrhizas also from mineral soil reveals a more complete picture of the whole ECM community.

In nature, ECM communities usually consist of a few common species and a large number of rare species. Given that ECM communities are highly diverse, patchily distributed and dominated by a few species, a complete determination of the species richness is impossible in most cases. Sampling effort and strategy have a major impact on the view we obtain of ECM community structure. It is important to be aware of that issue, especially when different ECM communities are compared. Sampling individual root tips randomly instead of studying bulk samples is recommended, because root-tip density of samples usually varies, affecting the number of observed species (Taylor 2002).

ECM species can be identified by morphological and anatomical characters (Agerer 1991). However, identification or grouping of ECM species in this way may be difficult and experience is needed. Sequencing of the internal transcribed spacer (ITS) region of rDNA is now commonly used for identification of ECM fungi (Dahlberg 2001, Horton and Bruns 2001). Although molecular techniques are a less subjective way to identify ECM fungi than morphotyping, they are not trouble-free. Identification of sequences can simply be based on sequence similarities. However, definition of ECM species by sequence similarities is complicated by the fact that the level of intraspecific ITS sequence variation differs between ECM fungi; e.g., ITS sequences in *Cortinarius* species are highly similar (Glen et al. 2001, Frøslev et al. 2005) whereas *Cantharellus* possess a strongly divergent ITS region (Tedersoo 2007). Contamination and artifacts are always risks in PCR amplification affecting directly to the quality of nucleotide sequences. Identification of sequences by comparing them to the sequences deposited in public nucleotide databases may be problematic, since taxonomic coverage of databases is limited. Only part of the soil microbes are represented in databases and many sequences are unidentified. Probably the most serious drawbacks of public databases are mistakes in sequences and in their identifications (Nilsson et al. 2006). To help overcome these problems in mycorrhizal studies, the UNITE database (<http://unite.ut.ee>) was created by Nordic-Baltic researchers (Kõljalg et al. 2005). In January 2008, the UNITE database contained 2511 well-annotated and trustworthy fungal ITS sequences from 118 genera.

Sequences are mainly derived from ECM fungi. The need for well-annotated sequences of other fungal groups is evident too.

1.2.4 Extraradical mycelium of ectomycorrhizal fungi

The extraradical mycelium of ECM fungi may form as much as 80% of the total ECM fungal biomass in boreal forest soil (Wallander et al. 2001). A remarkable proportion of the photosynthesized carbon allocated to the tree roots is retained in the extraradical fungal mycelium. Wu et al. (2002) estimated that 24% of ^{14}C -photosynthate was allocated to ECM extraradical mycelium of *Pinus densiflora* seedlings. Although the value was assumed to be an overestimate, the result confirmed that extraradical mycelium can be a significant sink for plant photosynthates.

The extraradical mycelium of ECM fungi consists of hyphae and rhizomorphs that emanate from the mantle surface into the soil. ECM mycelium increases the area of nutrient absorption by exploring vast areas around roots and by reaching soil pores that are too small for the roots to enter. It has been estimated that a tree root with extending ECM mycelium can exploit a 1000-fold larger soil volume than a non-mycorrhizal root (Rousseau et al. 1994).

ECM fungal species vary considerably in terms of the distribution and patterns of the extraradical mycelium, which may consist of only a few, if any, emanating hyphae or may comprise a dense mat of mycelia and reach various distances (Agerer 1987-1998, Agerer 2001). Hydrophilic fine hyphae are mainly responsible for nutrient and water absorption, whereas hydrophobic hyphae and rhizomorphs are responsible for nutrient uptake at greater distances (Unestam and Sun 1995). Rhizomorphs may emanate tens of centimeters from the mantle surface, and they are suitable for quick transportation of resources. Given that extraradical mycelium is mainly responsible for nutrient extraction and mobilization, the structural variation of mycelium is likely to represent functional differences between fungi. Agerer (2001) classified ECM fungi according to the amount and growth of hyphae and rhizomorphs into different exploration types, which may represent distinct foraging strategies: contact exploration type in genera *Lactarius*, *Russula* and *Tomentella*, short-distance type in *Cenococcum* and *Tylospora*, medium-distance type in *Amphinema*, *Dermocybe*, *Piloderma* and *Thelephora* and long-distance type in *Boletus* and *Paxillus*. Ectomycorrhizal fungal species producing copious mycelium can explore and acquire resources from wide areas and therefore they may represent the most beneficial species. On the other hand, a large proportion of plant-derived carbon is invested to the extraradical mycelium and therefore ECM fungi producing the most extensive mycelium are likely to be also the most expensive for trees. Thus, it appears that the amount and differentiation of ECM mycelium has a crucial role for resource balance and vigour of a tree.

Extraradical mycelium of ECM fungi can connect roots of the same or different plant species together forming in the forest soil the so-called “the wood-wide web”, i.e., “the common mycorrhizal network” (Simard and Durall 2004). The simplest mycorrhizal network is formed by a single ECM fungus genet, which connect two conspecific trees. As several different ECM fungal and tree species are involved, the complexity of common mycorrhizal network increases. The common mycorrhizal network has been proposed to mediate carbon and nutrient transfer between root systems (Simard et al. 1997, Wu et al. 2001). Seedlings or trees in shadow may thus be supported by surrounding trees as they all are connected to the common mycorrhizal network. Shared mycorrhizal networks are also hypothesized to affect succession and competitive interactions of trees (Horton and Bruns 2001, Simard and Durall

2004). Although the common mycorrhizal network seems to exist in nature, its functioning and ecological relevance is not clear (Simard and Durall 2004, Taylor 2006).

1.2.5 Sampling ectomycorrhizal mycelium

Despite its ecological relevance, ECM mycelium is not yet well explored in situ. This is mainly due to practical problems, as it has been difficult to distinguish ECM hyphae from hyphae of saprotrophs or other fungi growing in the forest soil. Composition of ECM fungal species can be studied by extracting fungal DNA from soil and by using sequencing or molecular fingerprinting techniques for identification (Landeweert et al. 2003, Koide et al. 2005, Genney et al. 2006). This is, however, a laborious approach. Due to the polyphyletic nature of ECM fungi, specific primers can not be developed and therefore investigation can not be restricted to ECM fungi.

Few years ago Wallander et al. (2001) introduced in-growth mesh-bags that can be used for separation of ECM mycelial growth in the field. The mesh bags are filled with acid-washed quartz sand and buried into the soil. The small mesh size of bags prevents penetration by plant roots. Unlike saprotrophic fungi, ECM fungi readily colonize inorganic sand as they obtain carbon from host trees. Studies have shown that after one growing season, the bags are mainly colonized by ECM fungi and the proportion of non-mycorrhizal fungi is minor (Wallander et al. 2001, Kjølner 2006). It has been supposed that the mycelial community shows again a divergent picture of the soil ECM fungal community compared to those based on ECM root-tips below-ground and sporocarps above-ground (Koide et al. 2005, Genney et al. 2006, Kjølner 2006). However, more studies are needed to evaluate the relation between the distribution of ECM fungal mycelium, root tips and sporocarps.

1.3 Needle litter decomposers

1.3.1 Needle decomposition in boreal forests

Forests cover 66% of land area in Finland (Peltola 2006). Forest litter comprises dead leaves, needles, twigs, branches, roots and remains of animals and microbes. The major part of the litter that falls onto the forest floor is leaf material (Dix and Webster 1995). In northern boreal forests, yearly needle litter fall by Norway spruce is around 2 t ha⁻¹ (Nilsson and Wiklund 1992). The decomposition of the litter is slow due to the cold climate. For example, Berg et al. (1982) found in central Sweden, 75% weight loss of Scots pine needle litter after 5 years. Hence, nutrients can be retained in northern boreal forest soils for years. The decomposition of needle litter is an ecologically relevant process due to its importance for nutrient cycling, energy transfer and consequently, for the function of the whole forest ecosystem. Knowledge about the factors affecting the decomposers and their activity is thus necessary.

During needle decomposition, easily decomposable sugars, amino acids and organic acids are consumed quickly followed by the slower degradation of cellulose and hemicellulose and finally, after many years, degradation of recalcitrant lignin. The decomposition can be divided into two phases (Berg and Staaf 1980, Berg 1986). In the first phase, decomposition is regulated by the concentration of nitrogen and other nutrients limiting the microbial activity as well as concentrations of easily degraded soluble material. In the later phase, lignin and lignified carbohydrates remain and the decomposition rate is regulated by factors affecting lignin decay.

A simple way to estimate the decomposition rate *in situ* is to enclose needles in a litter bag and determine the remaining litter mass after a certain period in the field. Although the process of decomposition is relatively well known, little is known about the fungal communities decaying needle litter in boreal forests.

1.3.2 Litter decomposers in boreal forests

Litter decomposition is carried out by soil animals and microbes. The role of soil animals is mainly to mix and fragment litter, whereas bacteria and fungi are mainly responsible for the decomposition process. In the boreal forest, fungi have a key role in litter decomposition and nutrient recycling due to their acidity tolerance and release of enzymes (Killham 1994, Dix and Webster 1995).

The community of fungal decomposers changes during the decomposition process and different functional groups dominate at different stages. Pioneer decomposers are weak parasites and so-called sugar fungi, which cannot utilize cellulose. Sugar fungi are mainly members of Deuteromycota and Zygomycota. After dissolved sugars are used, secondary sugar fungi and cellulolytic fungi appear in succession. They are mainly members of Ascomycota, Deuteromycota and Basidiomycota. Later basidiomycetes are the main colonizers of decomposing litter, as they are able to break down lignin polymers. The fungi capable of litter decomposition in the later phase have a pivotal role in nutrient cycling in boreal forests, because most of the nutrients remain stored in the needle litter until their action (Berg 1986). Yet, the knowledge of the identity of fungal species decaying needle litter in boreal forests is poor.

Living coniferous needles are commonly inhabited by endophytic ascomycetes, which do not show any external signs of their presence or cause any symptoms of disease (Wilson 1995). Endophytes are often found in samples taken from the litter layer (Mitchell et al. 1978, Livsey and Barklund 1992, Lindahl et al. 2007) and they are also thought to participate in initial decomposition of needle litter (Livsey 1995, Müller et al. 2001). Many studies agree that *Lophodermium piceae* is the dominant endophyte in Norway spruce needles (Barklund 1987, Sieber 1988, Livsey 1995, Müller et al. 2001). The role of *L. piceae* as a decomposer is, however, unclear (Müller et al. 2001).

The needle decomposers and the process of decomposition are affected by various abiotic and biotic factors. Environmental factors, most importantly temperature and moisture, and quantity and quality of litter are primary determinants of the decomposition rate (e.g. Murphy et al. 1998, Wilkinson et al. 2002, Aneja et al. 2006, Aneja et al. 2007). High levels of nutrients (e.g. nitrogen) and low lignin content predicts a high decomposition rate (Melin 1928). The decomposition rate may also be affected by soil abiotic and biotic properties, and by interactions between soil organisms (Koide and Wu 2003). Providing that endophytic fungi have a significant role in needle decomposition, the factors controlling endophytic colonization may also be relevant for the decomposition. Commonly, endophytic colonization is influenced by microclimatic conditions, age of the needle and host species (Carroll et al. 1977, Carroll and Carroll 1978, Hata and Futai 1996). The susceptibility to endophytes may also depend on host genotype (Todd 1988, Saikkonen et al. 2003). Moreover, the decomposition is affected by the presence of soil animals (Dix and Webster 1995). The mechanical breakage by soil animals increases the surface area available for microbial attack. Fragmented litter is further wetter and more compacted, and it supports a larger animal population than intact litter. Fecal pellets of soil animals are rich in nitrogen and thus the presence of soil animals also enhances microbial growth by improving nitrogen supply (Teuben and Verhoef 1992).

Litter quality differs significantly among tree species, particularly among conifers and deciduous trees. Thus species identity shapes the community structure of decomposers (Aneja et al. 2006, Aneja et al. 2007). Although tree species composition may affect decomposers and their performance, it seems that the diversity of litter does not necessarily increase decomposition rate (Wardle et al. 1997, Madritch and Cardinale 2007). Similarly, there is little evidence that decomposer richness enhances decomposition rate, implying that decomposition is not sensitive to change in biodiversity, because there is redundancy in soil decomposer communities (Andrén et al. 1995, Griffiths et al. 2000, Liiri 2001, Setälä and McLean 2004). On the other hand, importance of the fungal diversity to the decomposition may increase under changing environmental conditions (Toljander et al. 2006) and species that are functionally similar, but react to environmental changes differently, may increase stability. Species can be redundant only in certain circumstances (Chapin et al. 1997), so it is understood that diversity of decomposers improves resilience and resistance (Griffiths et al. 2000).

1.4 Multitrophic interactions between trees and soil microbes

1.4.1 Boreal forest soil as a habitat for microbes

Boreal forest soil is the habitat for a complex microbial community. Soil consists of various components and aggregates of mineral particles, organic residues and organic matter in various stages of decay, completed with dissolved minerals, soil water, gases, plant roots and soil organisms. In boreal forests the dominant soil type is podzol. Podzol soils are highly stratified and soil horizons are clearly distinct. Under the upper litter layer is the humus horizon, which is rich in organic matter and high in microbial biomass. Underneath the organic layers is the nutrient-poor eluvial horizon, underlain by illuvial horizon and parental soil. The natural soil environment changes constantly along with climate and with the action of plant roots and soil organisms. The spatially and temporally heterogeneous mixture of various microhabitats harbours a diverse community of soil organisms.

1.4.2 Effects of trees on soil microbes

At the local scale, soil biodiversity may be affected by interactions within trophic levels or by direct trophic interactions. On a larger scale, the presence of plants, plant species composition and diversity, mixture of plant litter types, understorey vegetation and above-ground interactions all influence soil ecosystems (Wardle 2006). Accordingly, in boreal forests, trees affect directly and indirectly the structure, biomass and activity of soil microbial communities by their root exudates and root activities, by their litter deposition and by their influence on microclimate and understorey vegetation.

In photosynthesis trees fix carbon that enters the soil as litter material, dead roots and root exudates, including sugars, amino acids, organic acids, fatty acids and enzymes (Grayston et al. 1996). The assimilated carbon compounds not only change the soil chemistry but above all, provide the soil ecosystem with energy. Thus, the carbon derived from above-ground photosynthesis drive the activity in the soil microbial community and is the basis for various microbiological interactions and processes. The factor most limiting the microbial growth in soil is the presence of readily available carbon compounds (Wardle 1992). A finding after tree girdling showed that a key driver of soil respiration in the boreal forest is the flux of current assimilates (Högberg et al. 2001).

Trees can also affect the structure of soil microbial communities and their activity through the litter deposition. The amount and quality of above-ground litter differ between tree species, as discussed in paragraph 1.3.2. Similar to above-ground litter, the amount and quality of below-ground litter may affect soil microbes. Moreover, the presence of tree canopies controls light, temperature and moisture conditions of the soil, thereby indirectly affecting the soil ecosystem. The effect of trees on soil abiotic conditions partly determines the composition of understorey vegetation, which further has an impact on the soil habitat and inhabitant microbial community (Malmivaara-Lämsä and Fritze 2003).

The rhizosphere is recognized the narrow zone of soil surrounded and influenced by roots (Hiltner 1908). In this zone, root exudates, organic breakdown products and uptake of water provide a habitat for microbes that differs from the bulk soil with regard to concentrations and forms of nutrients, soil structure, moisture and pH (Timonen and Marschner 2006). Thus the density and activity of microbes are generally higher in the rhizosphere than in the bulk soil (Rambelli 1973, Lynch 1990). Mycorrhizae increase the quantity and change the quality of carbon allocated below-ground (Rygielwicz and Andersen 1994). Moreover, the influence of plant-derived exudates, i.e., the rhizosphere effect, is extended by the ECM extraradical mycelium. As some of the plant-derived exudates are released through the ECM mycelium, the soil surrounded and influenced by the mycorrhizal fungi can be called the mycorrhizosphere (Rambelli 1973). Thus, fungal transportation of substrates via the mycelium not only responds to the soil heterogeneity but also creates it (Lindahl and Olsson 2004).

1.4.3 Interactions between soil microbes

It is likely that microorganisms in the mycorrhizosphere interact with each other in various positive, negative and neutral ways. Spatial distribution of ECM fungi varies in the same scale as soil microbial community structure (Pennanen et al. 1999, Lilleskov et al. 2004), indicating covariation between ECM fungal composition and bacterial distribution. In line with that, some microcosm studies have shown that the extraradical mycelium of ECM fungi affects the composition of the bacterial community (Timonen et al. 1998, Assigbetse et al. 2005, Frey-Klett et al. 2005). Interactions between ECM fungi and bacteria are mainly competitive, but positive interactions and so-called “mycorrhiza helper-bacteria” are also well documented (Garbaye 1994). Soil protozoa and various soil animals graze mycorrhizosphere bacteria and fungal mycelia. Thus, the ECM mycelium also offers a favourable habitat for soil fauna, and interactions between ECM fungi and rhizosphere bacteria can influence the distribution of soil protozoa (Timonen et al. 2004) as well as other soil fauna.

Inorganic nutrients are readily used by plants and soil microbes. The concentrations of dissolved nitrogen and other nutrients are low and competition for them is high. ECM fungi can have saprophytic capabilities and thus ECM fungi compete with saprotrophic fungi for organic nutrients as well. Competition between saprotrophic microbes and mycorrhizal fungi is proposed to decrease the rate of decomposition. This phenomenon is known as “Gadgil effect” according to Gadgil and Gadgil (1971, 1975; see also Koide and Wu 2003). The ability to obtain carbon compounds from host trees gives ECM fungi a competitive advantage against saprotrophic fungi. Most ECM species are, however, probably less efficient degraders compared to saprotrophic fungi (Schimel and Bennett 2004). Moreover, saprotrophic and ECM fungi are somewhat spatially separated in a boreal soil profile (Lindahl et al. 2007) and thus their habitats overlap only partly.

1.4.4 Summary

To conclude, a complex of direct and indirect links between trees and microbial communities is net involved in the boreal forest soil ecosystem. Trees affect soil microbes through root exudates, and they are in direct contact with ECM fungi, which further interact with mycorrhizosphere microbes, decomposers, and soil fauna. Another major route for the effect of trees on soil microbes is through the deposition of litter. Litter quality has a strong impact on the community of litter decomposers, which are linked with other soil microbes. Thus, trees directly and indirectly shape soil microbial communities; trees affect soil habitat and influence multitrophic interactions involved in the soil microbial community. As a result, the location of trees creates spatial patchiness in soil microbial communities. In boreal forest soil, for example, spruce trees are found to induce a characteristic patch size of 4–5 m in the microbial community structure (Saetre and Bååth 2000). Microbes are also affected by tree species identity (Priha 1999, Saetre and Bååth 2000). Yet, it is not known how intraspecific genetic and phenotypic variation of trees affects interactions in soil ecosystem and to what extent microbial communities differ under conspecific tree individuals.

2 AIMS OF THE STUDY

Diversity of plant species is known to have ecological relevance, and the effect of tree species on soil microbial communities has been noted. However, the influence of intraspecific variation of trees on soil microbial communities, and thus for the function and stability of forest ecosystem, is poorly known. In boreal forests, the diversity of tree species is low compared to the diversity of ECM fungal and other microbial species. If the genotype of tree species affects the structure of ECM community, it might reflect on the whole soil microbial community and, finally, partly explain tree growth and performance above-ground. On the other hand, tree individuality may affect the soil microbial community through litter deposition. The main hypothesis in this thesis was that in the boreal forest, the structure and function of the soil microbial communities differs among the conspecific tree individuals.

The specific aims in this thesis were (See Fig. 1):

1. To investigate the influence of Norway spruce clones with different growth rates on their associated ECM communities (I).
2. To study the effect of the Norway spruce clones on the extraradical mycelium of ECM fungi and the bacterial community associated with the fungal mycelia (II).
3. To compare the decomposition of needle litter from the Norway spruce clones, to investigate the role of needle identity on the community structure of fungal decomposers and to identify the fungal decomposers acting on needle litter (III).
4. To evaluate the role of clonal variation of Norway spruce in determining the microbial biomass, activity and community structure in the organic soil layer (IV).

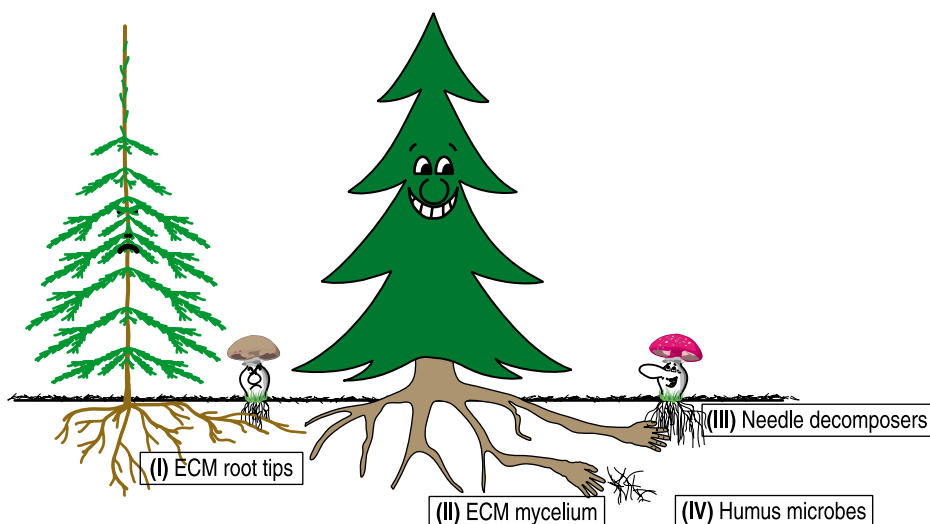


Figure 1. Components of the soil microbial community investigated in this thesis.

3 MATERIAL AND METHODS

3.1 Clonal field trial

The study site was a Norway spruce clonal field trial located in Pieksänmaa in central Finland (62°10'N, 27°16'E, altitude 101 m). Mean annual precipitation within the area is 640 mm and the mean monthly temperatures for January and July are -9°C and 16°C, respectively. The soil type is podzolized, fine sand moraine with an average humus depth of 5.9 cm. The site was occupied by Norway spruce (*Picea abies* L. Karst) with Myrtillus type (MT) understorey vegetation (Cajander 1949) before clear-cutting in 1991.

The Norway spruce clonal trial was established in 1994 by the Foundation for Forest Tree Breeding. The clones were generated in spring 1992 by rooting cuttings taken from seedlings of a few years old and maintained under similar conditions in peat pots. The 1-ha trial was laid out as a randomized block design with three replicate blocks all containing one plot of each spruce clone (Fig. 2). A plot (6 x 6 m) supported 9 cuttings planted 2 m apart from each other. Altogether the clonal trial consisted of 93 treatments. The height of the trees was measured in 2003 and 8 healthy clones were selected for this study (Fig. 2). Four of the clones were named as slow-growing and four as fast-growing according to their heights (Fig. 3). Maternal trees originated in southern Finland, Estonia and Germany (see Table 1 in paper I). The present understorey vegetation is dominated by *Festuca ovina*, *Calamagrostis arundinacea* and *Vaccinium vitis-idaea*.

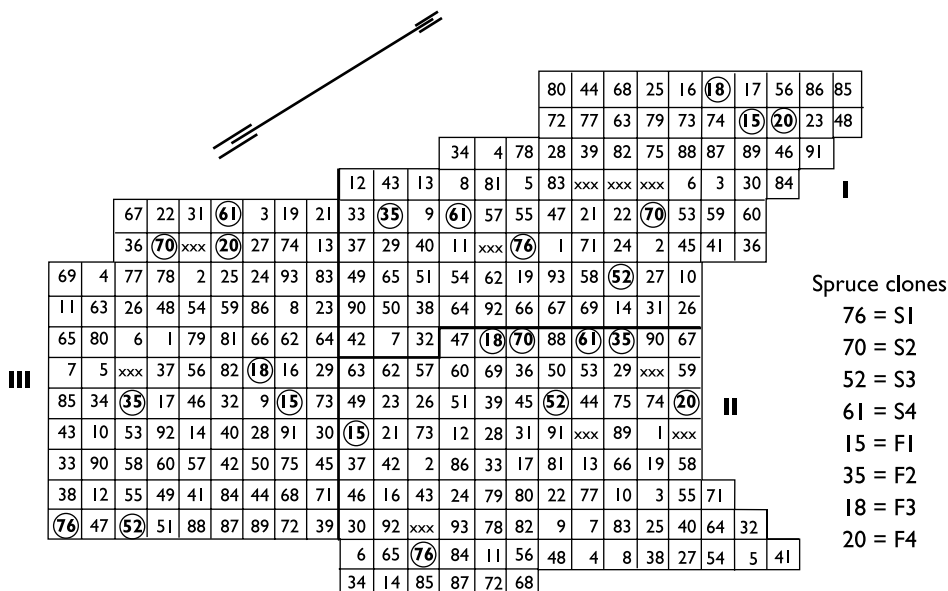


Figure 2. Experimental design at the clonal field trial of Norway spruce. The spruce clones selected for this thesis (S1–S4, F1–F4) are marked.

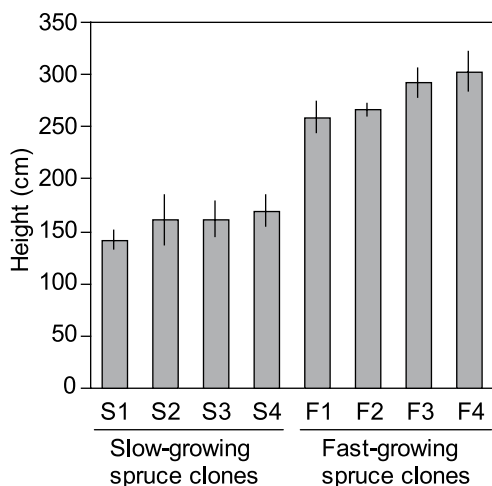


Figure 3. Height (mean \pm SE) of the Norway spruce clones.

3.2 Overview of the study

First, I investigated the ECM community in root tips of the Norway spruce clones (I). Root samples were collected from soil cores taken from each study plot. Roots were washed in tap water, cut into fragments and 10 ECM root tips per soil core (5 root tips from the organic and 5 from the mineral soil layer) were collected randomly under the dissection microscope. ECM root tips were identified and ECM community structure was compared among clones. At the same time when ECM root tips were collected the number of ECM root tips per length of fine root (ECM density), length of the fine root (<2 mm) in a soil core (fine root density) and dry biomass of fine root (fine root biomass) were determined for organic and mineral layer of each soil core.

Second, I looked into the ECM fungal community in extraradical mycelium harvested underneath each spruce clone with fungal in-growth mesh bags as described by Wallander et al. (2001) (II). The amount of mycelium and the structure of ECM fungal community were determined and compared among the differently growing spruce clones. The structure of the bacterial community associated with the ECM mycelium was also determined.

Third, I studied needle litter decomposers and decomposition rate among the spruce clones (III). Needles were incubated two-years *in situ* and *in vitro*. The *in situ* incubation showed fungal decomposers and needle mass loss in the field, whereas the *in vitro* incubation revealed the potential of endophytic fungi to act as decomposers after the early stages of the needle decomposition process.

Fourth, I studied the activity and community structure of microbes in the F/H layer (in this study humus refers to sieved soil from F/H layer) underneath the slow- and fast-growing spruce clones (IV). Soil core samples were collected and several analyses were performed to elucidate the structure and activity of microbial community. Soil chemistry, needle elemental concentrations and understorey vegetation of spruce clones were also studied to get a view of the factors that may have had an influence on microbial communities. The analyzed needles were the same as those used in study III.

3.3 Biological and chemical analyses

3.3.1 Introduction

The list of analyses and methods used in this thesis is presented in Table 1. Detailed descriptions of the procedures and references are given in the articles I–IV; here is given only a short overview of the analyses.

3.3.2 Microbiological analyses

Fungal community structures were analyzed by molecular methods: DNA extraction coupled with polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), cloning and sequencing. The DGGE procedure is based on the electrophoresis of PCR-amplified DNA fragments in polyacrylamid gels containing a gradient of increasing chemical DNA denaturants. As the fragments migrate in the gel, a transition of helical to partially melted molecules occurs and migration of the molecules halts. The melting temperature is sequence-specific and thus fragments with different base-pair sequences stop migrating at different positions in the gel. Band profiles can be visualized by staining and UV-transillumination.

The target region in molecular analysis was internal transcript (ITS) region of ribosomal DNA (rDNA). The ITS region lies between the 18S rRNA and 25S rRNA genes and it consists of two noncoding spacers, ITS1 and ITS2, which are separated by the 5.8S rRNA gene. The interspecific variation in the ITS region is high; it is the most commonly used area in fungal taxonomic studies and was therefore selected as a target region in this study.

The analysis of fungal decomposers was based on the ITS region of ribosomal RNA (rRNA). Since DNA can be isolated from dormant or dead cells, DNA-based analysis does not permit discrimination of metabolically active and inactive organisms. Metabolically active cells, however, constantly synthesize RNA, which is rapidly degraded in inactive cells. In bacterial cells, rRNA content correlates directly with cellular activity and growth rates. A similar relationship has not yet been proved for fungal samples and therefore the amount of fungal rRNA can not be used as a measure of fungal activity in a sample. Nevertheless, the fungal community structure inferred from RNA diversity represents the metabolically active part of the community and consequently the rRNA approach was ideal for investigation of needle decomposers. In this method, the complementary DNA (cDNA) was synthesized by reverse transcription of the extracted RNA to cDNA, which was further PCR-amplified and thereafter used in analyses.

Needle endophytic fungi were studied by cultivation as well as non-cultivation based molecular methods. Furthermore, the occurrence of the common endophytic fungus *Lophodermium* sp. in needle litter was estimated by counting the percentage of needles with black zone lines. Zone lines are characteristic to *Lophodermium* sp. and they are understood to be an antagonistic reaction against other fungal species or strains.

The structure of the microbial community in the organic soil and ECM mycelium was analyzed by the phospholipid fatty acid (PLFA) method. PLFAs are found in the cell membranes of all living microorganisms, but in different compositions. PLFAs turn over rapidly on cell death and they are not found in storage products. Therefore, variation in PLFA patterns reflects differences in microbial community structures and the composition of PLFAs indicates the types of bacteria present.

In PLFA analysis, lipids were extracted from the soil and mycelial samples with chloroform-methanol-citrate buffer mixture, and separated into neutral lipids, glycolipids and

phospholipids on a silica column. PLFAs were then subjected to mild alkaline methanolysis, and the fatty acid methyl esters were detected by gas chromatography. Peaks were quantified by comparing to the internal standard, and identified. Fatty acids are described as the number of carbons in the chain followed by a colon and then by the number of double bonds. ω precedes the position of the double bond from the methyl end of the molecule. The prefixes *i* and *a* indicate iso- and anteiso branching, *br* indicates unknown branching and *cy* indicates a cyclopropane fatty acid. *Me* refers to the position of methyl group from the carboxyl end of the chain. C15:1 indicates that the PLFA has 15 carbon atoms and one double bond, but the position of the double bond or branching position is unknown. Individual PLFAs were expressed as a mol% of the total amount of PLFAs in a sample.

The total amount of PLFAs was also used to indicate the total microbial biomass. Another method used to estimate total humus microbial biomass was substrate-induced respiration (SIR), where samples were incubated with glucose (20 mg ml⁻¹ soil water) for 2 h and evolved CO₂ was measured with a gas chromatogram and converted to C_{mic} by factor 0,389. The sum of PLFAs considered to be predominantly bacterial origin (*i*15:0, *a*15:0, 15:0, *i*16:0, 16:1 ω 9, 16:1 ω 7, *i*17:0, *a*17:0, 17:0, *cy*17:0, 18:1 ω 7 and *cy*19:0) was used as an indicator of bacterial biomass and 18:2 ω 6,9 was used to represent fungal biomass. The amount of ECM fungal mycelia harvested by in-growth mesh bags was estimated visually and as dry mass.

Microbial activity was measured as CO₂ evolved in 19–66 h. The growth rate of bacteria was estimated by [³H]-thymidine incorporation technique. Bacterial cells extracted from the humus were incubated with methyl-³H-thymidine and [³H]-thymidine incorporation into bacterial cells was measured as radioactivity in samples. The growth rate of fungi, on the other hand, was estimated as ¹⁴C-acetate incorporation into ergosterol. The soil suspension was incubated with ¹⁴C-acetate followed by extraction and measuring ergosterol content and its radioactivity. Acetate is a precursor of ergosterol, a molecule specific to fungi, and the amount of radioactivity detected in ergosterol can be used as an indicator of fungal activity. Decomposition rate was determined as loss of mass of needle litter. In the field, needles were incubated in litter bags placed on the litter layer. After two years, the remaining needles were weighted and the mass was compared to the initial mass.

3.3.3 Chemical analyses

Determination of dry weight was done after drying at 105°C over night and organic matter was calculated as the mass lost after burning at 550°C for 4 hours. Soil pH was measured from water suspension. Soil and needle total organic carbon and nitrogen content were determined by the dry combustion method. For soil elemental analysis, soil was extracted with 1.0 M ammonium acetate and concentrations of extractable cations were measured with an inductively coupled plasma emission spectrometer (ICP-ARL). Elemental concentrations of needles were determined by ICP-IRIS after wet ashing the samples by microwave in HNO₃ - H₂O₂ solution. Total acidity from soil acetate extractions was determined by titration with NaOH to pH 7.0. Cation exchange capacity (CEC) and base saturation (BS) of soil samples was then calculated.

3.3.4 Vegetation and microsatellite analyses

The understorey vegetation of the study site was investigated in July 2004. In each study plot, the coverage of plant species was visually estimated on 3 quadrats (1 m²), which were arranged along a northwest-southeast transect about 2 meters from each other.

Table 1. Analyses and methods used in the articles of this thesis.

Analyses and methods	Article
Microbiological analyses	
Fungal community structure	
Cultivation	III
DNA extraction and purification	I, II, III
cDNA synthesis	III
PCR	I, II, III
DGGE	I, II, III
Cloning	I, III
Sequencing	I, II, III
Black zone lines on needles	III
Microbial community structure	
Phospholipid fatty acids (PLFA)	II, IV
Total microbial biomass	
PLFA	IV
Substrate-induced respiration (SIR)	IV
Bacterial biomass	
PLFA	IV
Fungal biomass	
PLFA	II, IV
Extraradical mycelium in mesh bags	II
Microbial activity	
CO ₂ evolution	IV
Bacterial growth rate	
[³ H]-thymidine incorporation	IV
Fungal growth rate	
[¹⁴ C]-acetate incorporation	IV
Decomposition rate	
Mass loss of litter	III
Litter bags	III
Chemical analyses	
Dry matter (d.m.)	
+105°C	I, II, III, IV
Organic matter (o.m.)	
+550°C	II, IV
Soil pH	
Water suspension	IV
Total carbon and nitrogen in soil and needles	
Dry combustion	IV
Soil and needles elemental concentrations	
Ammonium acetate extraction (for soil)	IV
Wet ashing in HNO ₃ /H ₂ O ₂ (for needles)	IV
Soil cation exchange capacity (CEC)	IV
Soil base saturation (BS)	IV
Vegetation analysis	IV
Microsatellite analysis	I

Microsatellite analysis of spruce clones was performed to verify the origin of the ECM roots. Microsatellites are highly variable repetitive DNA sequences consisting of tandemly arranged short motifs. Due to a high mutation rate they are suitable markers for genetic fingerprinting.

3.4 Data analyses

A list of the statistical tests and multivariate analyses used in the thesis is presented in Table 2. Differences between the Norway spruce clones were analyzed by ANOVA (GLM) using block as a random factor, and followed by Tukey's test. The two growth performance groups (slow- and fast-growing spruce clones) were compared by Student's *t*-test or generalized linear model (GLM). A paired *t*-test was used to test differences between the organic and mineral soil layers in each study plot. Pearson's correlations were calculated between measured variables. When the assumptions of parametric tests were not met, transformations or corresponding non-parametric tests were used.

PLFA and ECM root-tip data were explored with principal component analysis (PCA) and detrended correspondence analysis (DCA), respectively. The scores of the spruce clones from PCA and DCA were further tested with ANOVA or Student's *t*-test. Binary matrixes from DGGE analyses were subjected to non-metric multidimensional scaling (NMS) to visualize the patterns of fungal community structures between the spruce clones. Similarly, NMS was used to analyze the understorey vegetation between the spruce clones. Canonical discriminant analysis (CDA) was used to determine how well the growth performance groups could be separated, given the values for the elemental concentrations of the needles, surrounding understorey vegetation (NMS dimension 1), soil chemical properties and humus microbial activity and community structure (PCA score 2).

Table 2. Statistical tests and multivariate analyses used in the articles of this thesis.

Data analyses	Article
Generalized linear models (GLM); ANOVA	I, II, III
Tukey's test	I, III
Student's <i>t</i> -test	I, II, IV
Paired <i>t</i> -test	I
Pearson's correlation	I, III
Kruskal-Wallis test	I, II
Mann-Whitney <i>U</i> -test	I, II, IV
Spearman correlation	II
Detrended correspondence analysis (DCA)	I
Principal component analysis (PCA)	II, IV
Nonmetric multidimensional scaling (NMS)	II, III, IV
Canonical discriminant analysis (CDA)	IV

4 RESULTS AND DISCUSSION

4.1 The main results

The influence of intraspecific variation of trees on soil microbial communities is poorly known. I investigated the communities of ECM root tips, ECM extraradical mycelium, needle litter decomposers and humus microbes underneath eight Norway spruce clones, showing considerable growth differences. Figure 4 summaries the main results. The community structures of ECM fungi (ECM root tips and ECM extraradical mycelium), bacteria associated with the mycelium, endophytic fungi and humus microbes differed among the spruce clones. However, the structure of fungal community inhabiting needle litter in the field did not differ between the clones. Clonal variation of spruce did not affect the loss of litter mass or the microbial biomass and activity in humus. Needle C:N ratio was higher with the slow-growing clones. Mosses were more dominant under the slow-growing clones, whereas coverage of grasses increased under the slow-growing clones. Any of the measured humus chemical properties did not differ among the spruce clones.

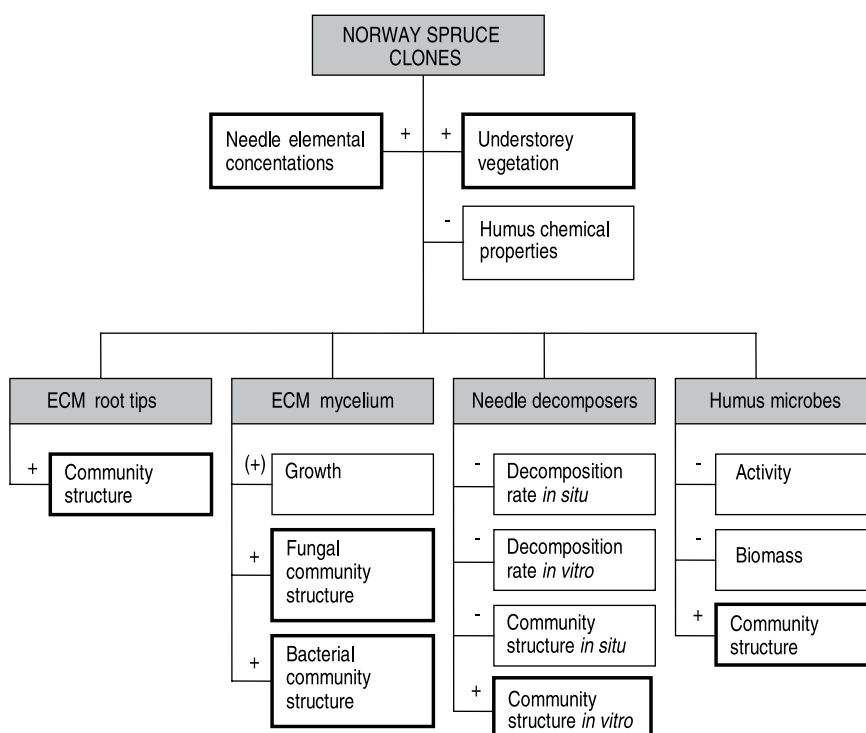


Figure 4. Responses of the studied soil microbial communities to the clonal variation of Norway spruce. Diagram also presents the variation in needle elemental concentrations, understorey vegetation and humus chemical properties measured. Explanation for the symbols: + indicates difference among fast- and slow-growing spruce clones, - indicates no difference, (+) indicates that differences were inconsistent.

4.2 Interactions between Norway spruce clones and ECM communities

4.2.1 Influence of spruce clones on ECM fungi

The influence of host tree individuality on the ECM community is largely unknown. I found that the structure of the ECM community (i.e. species composition and diversity) varied among the young Norway spruce clones (I, II). There was some difference in the ECM community structures within each of the growth performance groups suggesting that the genotype or some genotype-determined character of the spruce clones may have controlled the ECM formation and the composition of the ECM community (Fig. 4 in I). More significant, however, than the difference in the ECM community structures among the clones within each of group, was the difference in ECM composition and diversity between the growth performance groups (I, II). Both in the root tips and in the extraradical mycelium ECM fungal composition differed between the growth performance groups (I, II). The fast-growing spruce clones were associated with more diverse ECM root-tip community than the slow-growing spruce clones (I). In ECM extraradical mycelium the species diversity did not differ between the growth performance groups (II).

The fast-growing spruce clones had higher root-tip density (I). Nevertheless, ECM diversity did not correlate with the root-tip density (I). Same number of ECM root tips was randomly sampled from each spruce clone (I). This proved to be an effective sampling method, since although the number of identified ECMs was relatively small, the observed number of ECM taxa was 73% of the estimated richness. The difference in ECM fungal communities between the growth performance groups was observed not only in the ECM root tips but also in the ECM extraradical mycelium (II). Moreover, the actual difference in ECM diversity between the growth performance groups is likely to be higher than observed, because the proportion of the ECM community investigated in the fast-growing spruce clones was lower than that investigated in the slow-growing clones (Fig. 5 in I). Thus, the findings of this thesis indicate that the vigour of the spruce clone interacted with the structure of ECM community.

Nara et al. (2003) found a positive correlation between the size of host and the number of ECM fungal species when they investigated sporocarp production of ECM fungi during the primary succession on Mount Fuji, Japan. Similarly, a change in fungal richness and composition has been observed during the forest secondary succession (Visser 1995, Twieg et al. 2007). The proportion of rhizomorphic ECM types decreases after clear-cutting (Heinonsalo et al. 2007). It has been assumed that species typical for mature forests decrease, because they require hyphal contact and high root density to persist (Kranabetter and Friesen 2002). Recolonization by “late-stage” fungi takes years via expansion of vegetative mycelia. “Pioneer” fungi, on the other hand, are able to spread effectively by spores. Above all, they may be better adapted to the environment at the beginning of the forest succession with regard to soil physical and chemical condition and carbon supply (Deacon and Fleming 1992, Kranabetter and Friesen 2002). In this study, many late- and multistage fungi, such as species of Russulaceae and Boletaceae, were exclusive to the fast-growing spruce clones, whereas pioneer fungi and ascomycetes dominated the ECM communities underneath the slow-growing clones (I, II). However, the difference in ECM communities between the growth performance groups was not attributable to differences in the availability of the inoculum, because the spruce clones were randomly organized in three blocks at the 1-ha study site and dispersal of the ECM fungal inoculum was not restricted. Similarly, the randomized study design ensured that initial soil properties should not have caused the observed difference in the ECM communities under

the spruce clones. Therefore, it may be concluded that phenotypes and/or genotypes of the spruce clones shaped the ECM community in the study site.

The photosynthesis and carbon flow below-ground were not measured but it is likely that they were higher with the fast-growing clones, which are, on average, twice as tall as the slow-growing clones. It is known that carbon supply has a significant influence on ECM fungi (Högberg et al., 2001). It has been proposed that the carbon demand varies among ECM fungi, and pioneer fungi require less carbon than late-stage fungi from their host tree (Colpaert et al. 1996, Deacon and Fleming 1992). This could explain why particular ECM taxa were restricted to the fast-growing spruce clones. Even though it is not well confirmed that late-stage fungi require more carbon from their host than pioneer fungi, several experiments have showed that defoliation changes the ECM species composition and decreases ECM diversity (Saikkonen et al. 1999, Cullings et al. 2001, Kuikka et al. 2003). Following partial defoliation, the carbon supply per ECM root tip decreases and the consequent dramatic change in carbon flow may affect the structure of ECM community. The fast-growing spruce clones investigated in this study have more short roots than the slow-growing clones (I). Therefore it is unknown whether the relative amount of carbon per ECM root tip is higher with the fast-growing spruce clones than with the slow-growing clones. Nevertheless, it seems that quantity and/or quality of carbon allocated below-ground may have contributed to the difference in the ECM community between the growth performance groups.

4.2.2 ECM communities and habitats created by the spruce clones

Trees change soil chemical, physical and biological attributes (Pennanen et al. 1999, Liski 1995) and ECM fungi are known to vary in their habitat preferences (Cairney 2005, Toljander et al. 2006, Iwa ski and Rudawska 2007). Hence, the host tree may also shape the composition of ECM community indirectly, through effects on the abiotic and biotic properties of soil habitat. The ECM community may, for example, respond to the quantity or quality of litter (Conn and Dighton 2000). In line with that, litter quality may have had some influence on the investigated ECM communities of the spruce clones, because variation in the needle elemental concentrations among the slow- and fast-growing spruce clones was observed (III). Otherwise, the depth of the organic layer (I), pH and other measured soil chemical properties (Table 3, III) did not differ significantly beneath the spruce clones, suggesting that the effect of the spruce clone via edaphic properties was not strong.

In forest soils, ECM communities are generally highly heterogeneous in their spatial distribution (Tedersoo et al. 2003, Koide et al. 2005, Genney et al. 2006). The results of this thesis suggest that spatial patchiness may be partly caused by tree individuals. ECM communities are not only horizontally heterogeneous but also, as many previous studies have demonstrated, vertically distributed in the highly stratified boreal forest soil (Heinonsalo et al. 2001, Landeweert et al. 2001, Rosling et al. 2003, Tedersoo et al. 2003). Soil layers differ in resource quality and quantity, microclimate and soil texture and this can explain vertical distribution in ECM fungal communities. Nevertheless, a major driving force for the vertical variation of ECMs is the stratification of tree roots. In the clonal field trial investigated in this thesis, the densities of both fine roots and ECM root tips were higher in the organic soil layer (I), as is usually found (Makkonen and Helmisaari 1998, Rosling et al. 2003). Despite the vertical variation in root distribution, the diversity of ECM communities did not differ between organic and mineral soil layers (I). However, ECM fungal community composition differed vertically (I). Species of the genera *Piloderma* and *Amphinema* were relatively more common in organic soil, whereas fungi belonging to the ascomycetes, in particular *Wilcoxina*

Table 3. Soil pH and organic matter content (o.m., expressed as % of dry matter), cation exchange capacity (CEC, expressed as mmol kg⁻¹ o.m.), base saturation (BS, expressed as % of Ca, Mg, K, and Na of CEC), C:N ratio and elemental concentrations (expressed as µg g⁻¹ o.m., except Ca is expressed as mg g⁻¹ o.m.) at the study plots of the slow- (S1–S4) and the fast-growing (F1–F4) spruce clones. The data indicate means ($n = 3$) ± SE.

	Slow-growing spruce clones				Fast-growing spruce clones			
	S1	S2	S3	S4	F1	F2	F3	F4
pH	4.6 ± 0.2	4.4 ± 0.2	4.5 ± 0.1	4.4 ± 0.2	4.7 ± 0.1	4.5 ± 0.1	4.3 ± 0.1	4.3 ± 0.1
o.m.	21 ± 3	24 ± 2	26 ± 3	25 ± 3	23 ± 3	21 ± 1	26 ± 2	32 ± 2
CEC	240 ± 30	260 ± 10	220 ± 0	250 ± 20	200 ± 10	220 ± 10	230 ± 10	260 ± 10
BS	13 ± 4	10 ± 3	15 ± 1	11 ± 2	16 ± 5	13 ± 2	11 ± 1	12 ± 3
C:N	23 ± 0	24 ± 1	23 ± 0	26 ± 1	22 ± 2	24 ± 1	26 ± 0	25 ± 1
P	63 ± 3	57 ± 8	91 ± 16	62 ± 9	92 ± 26	68 ± 7	72 ± 8	110 ± 30
K	720 ± 40	760 ± 70	790 ± 30	810 ± 40	800 ± 30	770 ± 60	740 ± 110	780 ± 10
Ca	4.0 ± 1.2	3.7 ± 1.1	4.9 ± 0.6	3.7 ± 0.6	4.8 ± 1.4	4.2 ± 0.5	3.8 ± 0.4	4.3 ± 1.0
Mg	740 ± 50	690 ± 90	770 ± 50	730 ± 70	660 ± 130	680 ± 70	620 ± 70	680 ± 20
S	160 ± 10	150 ± 20	180 ± 10	150 ± 10	180 ± 10	150 ± 10	150 ± 10	160 ± 10
Fe	67 ± 12	52 ± 9	64 ± 5	43 ± 7	69 ± 19	58 ± 12	47 ± 2	32 ± 1
Mn	330 ± 100	250 ± 150	430 ± 140	290 ± 160	440 ± 80	290 ± 100	320 ± 70	220 ± 80
Zn	7.2 ± 0.7	6.5 ± 1.9	8.4 ± 1.5	7.2 ± 1.9	8.1 ± 0.6	6.4 ± 0.9	6.9 ± 1.7	5.2 ± 0.7
Na	45 ± 2	49 ± 5	40 ± 2	45 ± 1	48 ± 5	42 ± 1	43 ± 6	46 ± 4
Al	45 ± 10	38 ± 3	34 ± 9	29 ± 2	45 ± 18	37 ± 12	26 ± 1	17 ± 5

sp. and *Phialophora finlandia*, favored mineral soil. This agrees with previous findings (Heinonsalo et al. 2001, Rosling et al. 2003, Iwański and Rudawska 2007) and suggests that the investigated ECM communities contained species with varying habitat preferences and functional capabilities.

4.2.3 Influence of ECM fungi on growth of spruce clones

I found a relationship between ECM fungal community structure and growth of the spruce clones (I, II). Van der Heiden et al. (1998) demonstrated that high diversity of arbuscular mycorrhizas improved plant productivity. Their study, however, has faced criticism that the positive relationship between fungal diversity and plant productivity was an artefact of experimental design and a consequence of sampling effect, where an increase in diversity increases the probability that the community contains the most productive species (Wardle 1999). More recently, Maherali and Klironomos (2007) investigated the relationship between arbuscular fungal diversity and plant productivity by an experimental system that aimed to reflect non-random, more realistic community assembly. Fungal communities were constructed from the same number of fungal species and left to develop. After one year of growth, the communities with highest realized species richness stimulated plant productivity

more than those with low realized species richness. The authors concluded that taxa derived from distinct evolutionary lineages could coexist because of reduced competition and that they could enhance ecosystem functioning because of functional complementarity. In addition to arbuscular mycorrhiza diversity, ECM diversity has also been found to vary with the growth of trees (Baxter and Dighton 2001, Nara et al. 2003), but the relationship seems to depend on context, such as site fertility (Jonsson et al. 2001). Increased nitrogen availability in spruce forests has been found to decrease the ECM species diversity and mycelial growth (Taylor et al. 2000, Nilsson and Wallander 2003), indicating that under improved nutrient conditions the benefit of ECM community is less relevant for the vigour of tree. In addition, functional characters of the fungal species and interactions between the species may affect the functionality of the ECM community. According to current ecological theories, multiple species carry out similar functional roles in ecosystems and some species are complementary (Hooper et al. 2005). Undoubtedly this pertains to ectomycorrhizas too, and more important than phylogenetic diversity may be the functional diversity of the ECM community (Perry et al. 1989, Dahlberg 2001). Previous studies have introduced techniques to measure functional abilities of ECM fungi in terms of enzymatic activities and water transport in the laboratory (Courty et al. 2005, Plamboeck et al. 2007). Although some functional properties can be measured *in vitro*, nothing is known about the true functionality of fungi in nature. Moreover, it is likely that only a limited range of fungal properties that are thought to be beneficial for the hosts have been identified and can be measured.

The extraradical mycelium of ECM fungi is important for host tree nutrient supply, because it effectively takes up nutrients and water and transports them to the host tree. On the other hand, the amount of the external hyphae affects the carbon cost to the host, because most of the fixed carbon allocated to the ECM fungus is retained by the extraradical mycelium (Rygielwicz and Andersen 1998). Thus, the cost-benefit ratio finally determines the utility of the ECM mycelium to the host. Species forming extensive extraradical mycelium and long rhizomorphs were more abundant among the fast-growing spruce clones (I, II). The proportion of ascomycetes characterized by a smooth mantle and no rhizomorphs was higher with the slow-growing clones (II). Thus, the extraradical mycelia of ECM fungi tended to be denser under the fast-growing than under the slow-growing spruce clones (II). Higher biomass of fine roots (I) and total carbon allocation below-ground may have contributed to the mycelial growth under the fast-growing clones without any positive feedback to the growth of the clones. On the other hand, the positive relationship between the mycelial density and the growth of the clones may suggest that the dense ECM mycelium under the fast-growing clones enhanced nutrient and water supply and improved the growth of the spruces. Fungi may have other beneficial abilities, such as production of oxidases, which compensate the minor external mycelium (Agerer 2001). The results of this thesis, however, indicated that species with extensive extraradical mycelium, such as those belonging to the Atheliaceae, might be beneficial for the trees as they were relatively more common under the fast-growing spruce clones (II).

In general, the phenotype of the tree is considered as a result of genotype and environmental factors. Mari et al. (2003a) showed that there is genetic variation in growth and nitrogen uptake in non-mycorrhizal *Picea abies* seedlings, but ECM colonization by *Laccaria bicolor* reduced the differences among the spruce families. Given that, in nature, trees are mycorrhizal and mycorrhizal fungi are mainly responsible for nutrient uptake, tree genotype alone does not directly determine the growth performance but mycorrhizal community has an influence as well. The structure and functionality of the ECM community, on the other hand, may partly depend on the host genotype, as shown in this thesis (I). In tree breeding, ectomycorrhizas are

almost ignored, despite their prevalence and importance for tree growth and vigour. In addition to the nutrient and water supply, ECM fungi affect roots traits, such as number of short roots and shoot/root dry weight ratio, (Rosado et al. 1994a, Karabaghli et al. 1998, Jonsson et al. 2001). Rosado et al. (1994a) observed that heritability of root morphology traits was higher with mycorrhizal seedlings. The conclusion was that the ability to form mycorrhizas should be included as a criterion in the selection of genetic material for tree breeding. In this thesis, spruce clone S3 had low ECM diversity and even though the density of fine roots and ECM short roots were high, this clone was one of the smallest (I). This finding may indicate further that more important than the amount of fine and short roots is their function, which is mainly determined by the structure of ECM fungal community colonizing the short roots.

Taken together, although the ECM fungal composition and diversity differed between the growth performance groups, this study does not reveal anything about the causality. Even though the ECM communities may not have caused the initial growth difference between the clones, there may be a positive feedback between the growth and ECMs of the fast-growing spruce clones.

4.3 Needle litter decomposers among Norway spruce clones

4.3.1. Fungal taxa inhabiting litter in the field

Despite the importance of needle litter decomposition in boreal forests, the identity of the fungi playing a role in needle decomposition *in situ* has been, until recently, poorly known. I identified by molecular techniques fungi inhabiting needle litter of Norway spruce clones (III). The most frequent identifications were *Lophodermium*, Pezizales, *Mycena*, and *Marasmius*. Lindahl et al. (2007) presented in their study of boreal soil fungi a long list of fungal taxa that were found from different soil layers. They found previously unknown ascomycetes within Helotiales and Dothideomycetes, some known saprotrophic fungi, such as *Mycena* and *Marasmius*, and endophytic fungi of *Lophodermium*, in the surface litter. Contrary to the general suggestion that ECM fungi share habitats and functions with the saprotrophic fungi (Read and Perez-Moreno 2003, Steffen 2003), Lindahl et al. (2007) demonstrated that ECM fungi inhabit deeper soil layers than saprotrophic fungi. This partly agrees with the findings presented in this thesis, as *Lophodermium*, *Mycena* and *Marasmius* constituted 40% of fungal community in the spruce litter decomposed two years in the field (III). Additionally, members of the Pezizales were frequently observed (III). Members of this order exhibit varied modes of nutrition from saprophytic to parasitic, with many as yet unstudied. However, the Pezizales also contain mycorrhizal species so this thesis does not totally affirm the spatial separation of the ectomycorrhizal and saprotrophic fungi. Many fungal sequences could not be identified to the species level, demonstrating that the knowledge of fungal litter decomposers is still limited.

It is likely that the fungi studied in article III represented metabolically active needle colonizers, because the detection and identification were based on the ITS region of RNA. The RNA-based identification seems a very promising method for analyzing litter decomposers, as it concentrates on the active part of the litter-colonizing fungal community. Previously it was assumed that the ITS region is not suitable for RNA-based analysis, because it is a non-coding region and excised after transcription (Hibbett 1992). In contrast, the study reported in article III together with the study by Anderson and Parkin (2007) demonstrated that the fungal ITS region can be detected in the total RNA pool and it is suitable for molecular

marker studies. Metabolically active fungal cells constantly transcribe RNA, and thus the amount of un-cleaved precursor RNA molecules is high in RNA pool. Therefore, the ITS region can be detected in metabolically active fungi. Moreover, detection of the ITS region of precursor RNA may be a stronger indication of metabolically active organisms, because the turnover rate of precursor RNA is likely to be higher than that of mature RNA (Anderson and Parkin 2007). Study III is apparently the first to analyze the fungal community colonizing spruce needle litter in the field by using the ITS region of precursor RNA as a marker. Fungal colonization in spruce litter has previously been detected by analyzing 18S rRNA RT-PCR products (Aneja et al. 2006, Aneja et al. 2007). From spruce needles decayed eight weeks in a beech-spruce forest, fungi of *Penicillium*, *Trichoderma* and *Leptodontidium* were mainly extracted (Aneja et al. 2006, 2007) and these are representative of sugar fungi, which were not detected in litter after the 2-year decomposition (III). The findings support a change in fungal community during the decomposition process and suggest that primary colonizers are replaced by secondary colonizers within two years. On the other hand, Aneja et al. (2006, 2007) did not observe endophytic fungi, which are already present when needles drop on the soil and which have been suggested to be initial decomposers (Livsey 1995, Müller et al. 2001). Drying of the needle material prior to the incubation in the field may have inactivated the endophytes so Aneja et al. (2006, 2007) were unable to detect any known endophytic taxa in their studies.

4.3.2 Endophytic fungi as decomposers

As often reported (Barklund 1987, Sieber 1988, Müller et al. 2001), *Lophodermium piceae* was the most abundant endophytic fungus in the intact green needles of Norway spruce clones (III). Interestingly, after two years of decomposition members of *Lophodermium* still colonized the needle litter in the microcosms as well as in the field (III). RT-PCR products of *Lophodermium* sp. were the first and second most common *in situ* and *in vitro*, respectively. The findings are in accordance with those of Lindahl et al. (2007) who frequently recorded DNA of *Lophodermium* sp. in the needle litter on the soil surface. In contrast, Müller et al. (2001) noted that in the microcosm incubation, *L. piceae* was replaced by other endophytic fungi in a few months. However, disappearance of *L. piceae* in the study by Müller et al. (2001) may have been a consequence of the incubation temperature, which was too high for its sporulation. In this thesis, the microcosm experiment revealed that endophytes were able to decompose as much as 40% of needle mass in two years (III). It was also found that the relative abundance of DGGE bands of metabolically active *Lophodermium* sp. correlated positively to the loss of litter mass (III). These findings together suggest that endophytic fungi and, in contrast to previous expectations, members of *Lophodermium* (Müller et al. 2001) have a pivotal role in the decomposition of needle litter. Moreover, it seems that endophytic fungi can be competitive against soil-derived fungi even after two years. Mass losses of 60–70% *in situ* and 35–45% *in vitro* suggest that the initial stages of the decomposition was past (Berg and Ågren 1984) and therefore the results in paper III propose that endophytic fungi are not only primary decomposers but are involved in the later stages of decomposition as well.

In addition to *Lophodermium* sp., other identified endophyte genera detected in the needle litter incubated two years *in vitro*, were *Mollisia*, *Lachnum*, *Phialocephala* and *Zalerion* (III). None of these fungal taxa was found in green needles but all are known to include fungal endophytes (Sieber 1989, Hata and Futai 1996, Bergemann and Garbelotto 2006, Ganley and Newcombe 2006, Menkis et al. 2006). Contamination of the microcosms from air thus seems unlikely. More like is that members of *Mollisia*, and apparently also *Lachnum*, *Phialocephala*

and *Zalerion*, grow slowly in the needles and therefore became detectable only after long incubation periods (Dix and Webster 1995). *Mollisia* sp. was also found as an active fungus in the field (III). However, *Mollisia* sp. was infrequent *in situ* while it was dominant *in vitro*. Therefore, in the field the growth of *Mollisia* may have been restricted by competitive soil-derived saprotrophic fungi. Lindahl et al. (2007) found members of *Mollisia* from surface litter and also from fragmented litter and deeper in the soil profile. *Lophodermium* sp., in contrast, was restricted to the surface litter (Lindahl et al. 2007). Thus, it can be concluded that the proportion of *Lophodermium* sp. reduces during the succession of decomposing fungi, whereas *Mollisia* may also decompose more decayed litter. In addition, in this thesis it was detected that in two years *Mollisia* sp. replaced *Lophodermium* sp. as a dominant active endophyte *in vitro* (III). This may indicate that the saprotrophic lifestyle is more important for *Mollisia* than for *Lophodermium* sp.

4.3.3 Response of the litter-colonizing fungi to clonal variation of spruce

Spruce clones did not affect the needle litter decomposition rate *in situ* or *in vitro* (III). The structure of fungal community inhabiting needle litter after two years decomposition differed between the growth performance groups *in vitro*, but not *in situ* (III). *In vitro* experiment *Lophodermium* tended to be more frequent with the fast-growing clones, whereas the slow-growing clones supported more endophytic taxa (III).

It has long been recognized that an important driver of decomposer communities and their activity is quality of plant litter, i.e., the structural and chemical traits of the litter. The level of nutrients (e.g. nitrogen) may have a major role in a first phase of conifer needle litter decomposition, whereas lignin content regulates the decomposition in later stages (Berg and Staaf 1980). Plant species identity may affect litter decomposers and decomposition rate through the substrate quality (Murphy et al. 1998, Wilkinson et al. 2002, Aneja et al. 2006, Aneja et al. 2007). As the quality of the litter is affected by its genetic pedigree, genetic variation among conspecific trees may also affect decomposition and nutrient fluxes (Madritch and Hunter 2002, Madritch and Hunter 2005, Silfver et al. 2007). Similarly, the spruce clones investigated in this thesis showed variation in needle elemental concentrations (IV) and C:N ratio of needles correlated negatively to the loss of litter mass in the field (III). However, differences in the C:N ratio of needles of the spruce clones were so small that the spruce clones did not differ significantly in mass loss of litter *in situ* or *in vitro*. The analyzed needles were sampled in autumn instead of winter when elemental concentrations are at more stable level, because winter time is not as optimal for the investigation of endophytic colonization (Sieber 1988).

Although the decomposition process can be affected by genetic variation of litter, the response of decomposing microbes to litter identity has received less attention. Silfver et al. (2007) observed that that genotypes of *Betula pendula* varied in loss of litter mass, but no effect of litter identity on soil bacterial and microarthropod communities was observed. The fungal community structure was not investigated by these authors. In this thesis, the community structure of fungal decomposers colonizing the litter in the field did not respond significantly to the clonal variation of the needles (III). In the microcosm where all fungi but endophytes were excluded, the difference in decomposer communities between the growth performance groups was more obvious (III). Thus, the hypothesis that Norway spruce clones affects the decomposer community and decomposition rate through the quality of needle litter was supported only *in vitro* where the effect of environmental factors were mainly excluded.

However, the result should be assessed taking into account the small number of analyzed needles.

Although it has been shown previously that resistance of a host tree to endophytic fungi has a genetic component (Todd 1988, Saikkonen et al. 2003), the proportion of needle segments colonized initially by endophytes did not differ between the spruce clones (III). However, after two years of incubation the structure of endophytic communities differed between the two growth performance groups of clones: more endophytic taxa colonized the litter of the slow-growing clones, whereas the relative abundance of *L. piceae* was higher on the fast-growing clones (III). This may suggest that endophytic fungal communities initially colonizing the green needles differed among the spruce clones, even though this was not established. In the field, the difference in the endophytic fungal occurrence between the growth performance groups was not significant at the end of the experiment, but this is understandable due to the presence of competing soil-derived microbes.

Endophytic fungi are usually considered to be in neutral or beneficial relationship to their host plants. However, it is also possible that endophytes are harmful to the host plant and the host constantly needs to spend resources for controlling their growth. Schults et al. (1999, 2002) and Schults and Boyle (2005) proposed that endophyte-host interaction is not neutral, but instead is balanced antagonism. If the balance between the endophyte and host is disturbed by either a decrease in plant defense or an increase in fungal virulence, endophytes become pathogenic and disease develops. In this regard, it is relevant that needles of the slow-growing spruce clones were colonized by more diverse community of endophytic fungi, whereas needles of the fast-growing spruce clones were mainly inhabited by *L. piceae* (III). The finding supports the assumption of the non-harmful role of *L. piceae* for green needles (Barklund 1987) and implies that the other detected endophytes conversely may not have been as beneficial, or they might have even reduced the host spruce vitality. It can be speculated that the tree genotypes favorable to *L. piceae* colonization were protected against harmful fungi and therefore grow better. Causalities between the endophytic colonization and the growth performance can not be investigated in this study, anyway.

4.3.4 Influence of the fungal community structure on decomposition

The trend of positive correlation between the number of fungal rRNA-derived DGGE bands and the *in vitro* loss of litter mass may indicate that increasing diversity of decomposer fungi enhanced the decomposition rate (III). This would agree with the controversial hypothesis of diversity and ecosystem functioning (Waide et al. 1999, Loreau et al. 2001), and support the assumption of the importance of endophytic fungi as needle decomposers. Comparable relationship between fungal diversity and mass loss was not observed in the field, where soil-derived competitors were present and environmental factors were not controlled.

Although the relationship between species diversity and ecosystem functioning has received much interest, few studies have investigated the impact of community structure of decomposers on litter decomposition. Setälä and McLean (2004) proposed that the increase of fungal diversity enhanced the decomposition rate only in species-poor communities, and that communities of fungal decomposers contained functional redundancy. In the same way, the endophytic fungal communities in decaying needle litter of the slow- and fast-growing spruce clones *in vitro* showed functional equivalency. The mass loss did not differ among the spruce clones despite the different endophytic fungal communities; the decomposition of the litter from the slow-growing clones was stimulated by a more diverse endophyte community

(see Figs 5c and d in III), whereas the more frequent occurrence of *Lophodermium* tended to enhance litter decomposition among the fast-growing clones (see Fig. 6 in III), and therefore no difference between the growth performance groups was observed in the loss of litter mass *in vitro*. The positive relationship between the relative abundance of *Lophodermium*-derived rRNA band and mass loss suggest the functional key role of *Lophodermium piceae* in needle litter decomposition (III). There has been much debate about the importance of species diversity in contrast to species composition in the context of ecosystem productivity (Aarssen 1997, Huston 1997, Wardle et al. 2004). The results of this thesis suggest that both the fungal diversity and species composition were relevant for the needle litter decomposition *in vitro*.

4.4 Effect of Norway spruce clones on the humus microbial community

4.4.1 Direct or indirect effects

It is known that spatial structure in soil microorganisms can be induced by trees (Pennanen et al. 1999, Saetre and Bååth 2000, Wilkinson and Anderson 2001). The humus PLFA profiles reported in paper IV imply that in monoculture stands, tree identity also explains the spatial patchiness of microbial communities in the humus layer: the PLFA profiles representing microbial community structure differed between the growth performance groups. Assuming that photosynthetic capacities differ between the growth performance groups, quantity of carbon allocated below-ground is likely to be higher with the taller clones than with the smaller clones. The result that the microbial biomass and activity were equivalent under the spruce clones still suggests that the structure of soil microbial community change more readily than the microbial growth (IV). In line with that, previous studies have shown that soil microbial community structure, but not basal respiration and microbial biomass, respond to forest succession (Merilä et al. 2002), soil fertility and moisture (Pennanen et al. 1999). The PLFAs that mainly separated the growth performance groups were C15:1, 17:1 ω 8, 20:0, 19:1b, 20:5, 20:4 and 18:2a (IV). Most of the same PLFAs have been associated to forest patches dominated by Norway spruce (Pennanen et al. 1999, Merilä et al. 2002), suggesting that they are associated with soil biota surrounding spruces.

4.4.2 Ectomycorrhizas mediating the influence of spruces

The ECM fungal communities differed under the two growth performance groups (I, II), and thus they may have made a contribution to the variation of the structures of humus microbial communities (IV). Differences in the PLFA profiles of the humus and ECM extraradical mycelium may be attributed to the fact that these two communities were, of necessity, sampled on different occasions (II, IV). On the other hand, dissimilar PLFA profiles may imply that the ECM fungi affected the humus microbial community indirectly. It has been estimated that in a mature boreal forest, ECM mycelium contributes one-third of soil microbial biomass (Högberg and Högberg 2002). In young forests, the proportion of ECM fungi in soil microbial biomass is probably less, and thereby PLFAs derived from the ECM mycelium or its associated microbes did not reflect the humus PLFA profile (II, IV). Moreover, it seems that in the investigated trial, ECM biomass did not constitute a notable proportion of humus microbial biomass, because fungal biomass did not differ between the growth performance groups although ECM mycelial growth did (II, IV). Thus, ECM communities may have affected the community structure of humus microbes indirectly through effects on mycorrhizosphere microbes.

Studies of plant-fungus-bacteria interactions in the mycorrhizosphere have thus far mainly focused on rhizobacteria that promote plant growth and on mycorrhiza helper bacteria that promote the establishment of mycorrhizal symbiosis (Garbaye 1994). The question of whether the bacterial community responds to the ECM mycelial composition has received less attention. Nurmiaho-Lassila et al. (1997) found that morphologically different bacteria were associated with *Suillus bovinus* and *Paxillus involutus* mycorrhizospheres of *Pinus sylvestris*. The findings of Timonen et al. (1998) confirmed this result. In this thesis, the ECM extraradical mycelium growing under the slow-growing spruce clones harboured relatively more PLFAs indicative of Gram-negative bacteria, whereas PLFAs indicative of Gram-positive bacteria were more typical for the ECM mycelium underneath the fast-growing clones (II). This result indicates that in the mycelium, fungal composition affected bacterial composition (II). Furthermore, the impact of the ECM community on mycorrhizosphere microbes may have modified the whole soil food web, which was made evident in the community structure of humus microbes (IV). An increase of eukaryotic PLFAs (e.g. 20:4) in the humus under the fast-growing spruce clones (IV) also suggests that protozoa or other soil fauna may have been attracted by ECM mycelium and/or mycelium-associated bacteria (Federle 1986). The altered diversity of the soil fauna may have induced changes in microbial community composition further.

4.4.3 Link through litter quality, root exudates and understorey vegetation

Litter deposition of spruce clones may have affected the differentiation of microbial communities in humus layer (IV). The difference in the initial elemental concentrations of the needles did not directly affect the community structure of fungi colonizing needle litter in the field (III). However, lignin content or other chemical and structural properties of needles that were not determined may have differed between the spruce clones. Hence, the quality of organic humus may have differed and caused differentiation of bacteria and other soil biota. Soil pH and C:N ratio are important factors influencing soil microbial community structure (Högberg et al. 2007). In the present study, soil pH, C:N ratio and all other determined soil chemical properties did not explain divergent soil microbial communities (IV). Nevertheless, rhizodeposition including root exudation and dead roots was not measured and its potential for contributing to microbial variation is also possible.

Besides ECM fungal communities, litter deposition and root exudates, understorey vegetation may have induced the difference in the structure of humus microbial communities under the two groups of clones (IV, Malmivaara-Lämsä and Fritze 2003). The proportion of mosses increased toward the fast-growing spruce clones, whereas grasses were more abundant under the slow-growing clones where light conditions were better (Table 4, Fig. 1 in paper IV). Grasses also have a higher pH optimum than mosses but, as soil pH was consistent, its contribution to variation in understorey vegetation was limited (IV). Differences in understorey vegetation under the two groups may have further affected soil microbial community structure through litter deposition and root exudates. The relative abundance of PLFA 16:1 ω 5 has often been found to be related to young forest and forest openings where grasses are abundant (Pennanen et al. 1999, Malmivaara-Lämsä and Fritze 2003). Grasses are associated with arbuscular mycorrhizal fungi and PLFA 16:1 ω 5 is assumed to be indicative of arbuscular fungi (Olsson et al. 1995). Despite variation in grass coverage, PLFA 16:1 ω 5 did not follow variation in grass coverage (IV) suggesting that variation in humus microbial communities between the growth performance groups was more likely to be caused by spruce clones or mosses than understorey grasses.

Table 4. Total coverage (%) of grasses, mosses and plant species at the study plots of the slow- (S1–S4) and fast-growing (F1–F4) spruce clones. The data indicate means ($n = 3$).

	Slow-growing spruce clones				Fast-growing spruce clones			
	S1	S1	S3	S4	F1	F2	F3	F4
Grasses	55	65	35	44	31	39	34	44
Mosses	23	22	33	42	56	39	43	40
<i>Rubus idaeus</i> L.	0.1	0.8	0.2	0	0.5	0.2	0.7	0.4
<i>Calluna vulgaris</i> (L.) Hull	0	0.4	0	1.1	0	0	0	0
<i>Vaccinium myrtillus</i> L.	0.1	0	0.2	0	0.2	0	0.1	0.1
<i>Vaccinium vitis-idaea</i> L.	3.6	1.8	1.6	7.3	3.6	5.3	9.1	3.7
<i>Epilobium angustifolium</i> L.	3.8	8.1	3.3	0.3	4.1	1.8	2.6	1.2
<i>Maianthemum bifolium</i> (L.) F.W. Schmidt	0.1	0.1	0.3	0.3	1.1	1.8	0.1	0.2
<i>Pyrola</i> sp.	0.2	0.3	0.1	0	0	0	0.1	0.1
<i>Rubus saxatilis</i> L.	2.8	1.4	8.1	2.1	1.4	3.8	0.2	0.1
<i>Solidago virgaurea</i> L.	0	0	0.1	0	0	0	0	0.3
<i>Agrostis capillaris</i> L.	0	0	4.6	0	0	0.3	0	0
<i>Calamagrostis arundinacea</i> (L.) Roth	6.2	8.4	9.0	6.4	4.2	3.9	5.1	3.8
<i>Festuca ovina</i> L.	21	25	7.2	17	5.6	16	9.8	31
<i>Dicranum</i> sp.	0.8	1.4	8.1	2.0	5.1	3.2	2.1	4.7
<i>Pleurozium schreberi</i> (Brid.) Mitt.	19	12	18	7.8	38	28	24	16
<i>Brachythecium</i> sp.	0	0	0.1	0	4.4	0	0	0
<i>Polytrichum</i> sp.	0.9	0.7	2.9	12	4.4	4.4	17	15
<i>Hylocomium</i> sp.	1.1	0	0	0	0	0	0	0

5 CONCLUSIONS

This thesis demonstrated that the microbial community living in the forest soil below Norway spruces is diverse and contains many unidentified species. Investigation of the relationship between Norway spruce clones and soil microbial communities showed that genetic and phenotypic variation of same-aged conspecific trees can create spatial patchiness in soil microbial community and influence biodiversity in boreal forest soils. Clonal variation of spruces may have affected the multitrophic interactions in soil biota, because changes were observed in the ECM community, and ECM mycelium associated bacteria as well as in humus PLFA profiles indicative of microbial communities. The hypothesis that the litter quality is a determinant of decomposer community structure was supported *in vitro* but not *in situ*, where the presence of soil-derived competitors and fluctuation in abiotic factors may have confounded the clone effect. Findings on needle decomposition suggest that needle endophytic fungi are important decomposers. However, a negative correlation between spruce growth and endophytic diversity raises a question about the nature of plant-endophyte association. Then again, the fast-growing spruce clones were associated with more diverse ECM community than the slow-growing clones. Although it seems that spruce clones have shaped the ECM community and other soil microbes, the mechanisms are uncertain. There may be a positive feedback between the growth of spruce clones and the structure of the ECM community but the hypothesis remains to be tested that initial growth performance is determined by the ability of a tree genotype to draw an optimal combination of ECM fungal partners from a diverse pool of potential symbionts.

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