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The Role of Plant-Fungal Interaction for the Soil Organic Matter Degradation in Boreal Forest Ecosystem

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THE ROLE OF PLANT-FUNGAL INTERACTION FOR THE SOIL ORGANIC MATTER DEGRADATION IN BOREAL FOREST ECOSYSTEM

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ACADEMIC DISSERTATION

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List of original publications

This thesis is based on the following publications:

I Sietiö O-M, Tuomivirta T, Santalahti M, Kiheri H, Timonen S, Sun H, Fritze H, Heinonsalo J. (2018) Ericoid plant species and *Pinus sylvestris* shape fungal communities in their roots and surrounding soil. New Phytologist 218:738–751 (doi:10.1111/nph.15040).

II Adamczyk B, Ahvenainen A, Sietiö O-M, Kanerva S, Kieloaho A-J, Smolander A, Kitunen V, Saranpää P, Laakso T, Strakova P, Heinonsalo J. (2016) The contribution of ericoid plants to soil nitrogen chemistry and organic matter decomposition in boreal forest soil. Soil Biology and Biochemistry 103:394–404 (doi:10.1016/j.soilbio.2016.09.016).

III Sietiö O-M, Adamczyk B, Santalahti M, Putkinen A, Sun H, Heinonsalo J. Restriction of plant photosynthetic carbon flow affect microbial community but does not shift the dominance from ectomycorrhizal fungi to saprotrophs in humus. *Submitted*.

The publications and manuscript are referred to in the text by the roman numerals.

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I Outi-Maaria Sietiö participated in planning the experimental work, performed DNA extraction from the roots, participated in execution of the density gradient ultracentrifugation, processed and analyzed the sequencing data, interpreted the data together with the co-authors, wrote the first version of the manuscript, and is the corresponding author.

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III Outi-Maaria Sietiö participated in planning the experimental work, participated in the sample processing, processed and analyzed the sequencing data, analyzed the GeoChip data, interpreted the results, wrote the first version of the manuscript, and is the corresponding author.

Abbreviations

CCA	Canonical correspondence analysis
CMN	Common mycelial network
DSE	Dark septate endophyte
ECM	Ectomycorrhiza
ERM	Ericoid mycorrhiza
Humus	Mixture of fermenting litter (O_F) and humified organic matter (O_H) layers of boreal forest soils
ITS	Internal transcribed spacer
OTU	Operational taxonomic unit
PAC	*Phialocephala fortinii* s.l.–*Acephala applanata* species complex
PCoA	Principal coordinate analysis
rDNA	ribosomal DNA
rRNA	ribosomal RNA
SOM	Soil organic matter
SIP	Stable isotope probing

Abstract

The boreal forests are significant sinks for carbon, and the majority of the carbon present in boreal forests is stored in the soils. Especially in the organic layers of the boreal forest soils, a significant amount of the carbon is stored as complex polymers, which are rather stable and have a long residence time. In addition, these soils are considered to be nutrient poor since important nutrients, such as nitrogen and phosphorus, are complexed with soil particles or stored in organic forms, which are inaccessible to the plants. For acquiring nutrients from the complex substrate, plants form mutualistic associations with fungi.

In boreal forest ecosystems, where above-ground vegetation is dominated by coniferous trees, shrubs and mosses, the most common fungi associated with plant roots are ectomycorrhizal and ericoid mycorrhizal. In return for the enhanced water and nutrient uptake, the plants provide energy to their fungal symbionts in the form of recently photosynthesized carbon. As it is predicted that the amount of atmospheric CO_2 levels will rise and the climate will warm, the growing season will be extended in the boreal zones and the input of photosynthetic C from the plants to the soil will increase. This increase in the input of photosynthates from the plants can accelerate the below-ground processes and enhance the degradation of the older, stable soil organic matter through a phenomenon called the "priming effect". Thus, a more thorough understanding of the dynamics of plant-fungal interactions and their importance to the functioning of the whole boreal forest ecosystem is essential when predicting ecosystem level responses to the changing environment.

This PhD thesis aimed to enlighten the effect of plant-derived photosynthetic carbon to the fungal community structures and soil organic matter decomposition. Two studies of this thesis are based on a laboratory scale microcosm experiment, and the third study is based on a multiyear field experiment. Fungal community structure was assessed using high throughput sequencing. In addition, the first study provided new insights on the root-associated fungal communities which utilized photosynthates directly from the plant, as well as their host preference via a stable isotope probing technique.

The common boreal forest plants, ericoid shrubs *Calluna vulgaris* (common heather), *Vaccinium myrtillus* (bilberry), and *Vaccinium vitis-idaea* (lingonberry), and the conifer *Pinus sylvestris* (Scots pine) shaped their root and surrounding soil associated fungal communities differently from each other, while they also shared many fungal species. In addition, the ericoid shrubs and their associated microbes had different effects on the soil chemistry and enzymatic activities compared to the conifer *P. sylvestris*. The results from the laboratory scale experiment described in the first two studies of this thesis indicate that ericoid plants have an important influence on fungal community structures and processes in boreal forest soils.

The manipulation of the carbon flow from the plant to the soil induced moderate changes to the fungal community structure in humus during a three-year field experiment.

According to the ecological theory known as the “Gadgil effect”, decomposition should be enhanced when saprotrophic fungi are alleviated from competition with ectomycorrhizal fungi. However, the results of the third study of this thesis did not support the Gadgil theory, indicating that the competitive outcome between the saprotrophic and ectomycorrhizal fungi is substrate dependent and these two fungal guilds have preference towards different ecological niches. In addition, the results suggest that the members of the soil microbial community are rather flexible and can adapt to temporary disturbances.

This PhD thesis provided further insights into the role of plants in determining the fungal community structure on their own roots and surrounding soils as well as shaping the soil chemical profile. The results underline that for predicting how the changing climate affects soil processes in the boreal forest ecosystem, more knowledge on plant microbe interactions and their impact on soil processes is needed.

Tiivistelmä

Boreaaliset metsät ovat merkittäviä hiilivarastoja ja suurin osa niihin varastoidusta hiilestä on maaperässä. Erityisesti maaperän orgaanisessa kerroksessa hiili on muuntunut hitaasti hajoavaksi humusaineeksi, joka muodostaa boreaalisen metsämaan pysyvimmän hiilivaraston. Koska metsämaassa vain pieni osa tärkeistä ravinteista on kasveille helposti hyödynnettävissä muodoissa, turvatakseen ravinteiden saannin kasvit muodostavat sienijuurisymbiooseja niiden juuristoissa elävien sienten kanssa.

Boreaalisissa metsissä havupuut ovat yleisiä ja metsän aluskasvillisuus koostuu pääasiassa varpukasveista sekä sammaleista. Havupuut muodostavat tyypillisesti sienijuurisymbioosin pintasienijuurisienten kanssa (ektomykorritsasienet) ja varpukasvit kanervasienijuurisienien (erikoidimykorritsasienet) kanssa. Vastineeksi turvatusta ravinteiden- ja vedenotosta, sienijuurisienet saavat energiansa isäntäkasveilta erilaisina hiiliyhdisteinä eli sokereina. Ihmisen toiminnan aiheuttaman kasvihuoneilmiön voimistumisen ja ilmakehän CO_2-pitoisuuden noustessa myös kasvukaudet pidentyvät ja sen myötä kasvien maahan allokoiman hiilen määrien on arveltu kasvavan. Helppokäyttöiset hiiliyhdisteet antavat mikrobeille mahdollisuuden tuottaa tavallista enemmän orgaanisen materiaalin hajotukselle välttämättömiä entsyymejä ja näin lisäävän myös vaikeasti hajotettavien hiiliyhdisteiden, kuten humuksen, hajotusta. Parempi tietämys kasvi-sieni-vuorovaikutuksen merkityksestä boreaalisen metsäekosysteemin toiminnalle on tärkeää, jotta voidaan arvioida muuttuvan ilmaston vaikutuksia maaperän hiilenkierrolle ja hiilivarastoille.

Tämän väitöskirjatyön tarkoituksena oli tutkia kasvien maahan syöttämien hiiliyhdisteiden merkitystä juuriston ja maaperän sieniyhteisön monimuotoisuudelle sekä maaperän hiilenkierrolle. Tämän työn kaksi ensimmäistä osajulkaisua perustuivat laboratoriomittakaavan mikrokosmoskokeeseen ja viimeinen monivuotiseen kenttäkokeeseen. Sieniyhteisöjä ja niiden monimuotoisuutta tutkittiin molekyylibiologisin menetelmin ja syväsekvensoinnilla. Lisäksi ensimmäisessä osajulkaisussa tunnistettiin isäntäkasvin (kanerva, mustikka, puolukka ja mänty) kanssa suorassa vuorovaikutuksessa olleet sienet hyödyntäen DNA:n isotooppileimaukseen perustuvaa tekniikkaa.

Tutkittujen boreaalisen metsän kasvien (kanerva, mustikka, puolukka ja mänty) juuristojen ja ympäröivän maan sieniyhteisöjen rakenteet erosivat toisistaan, mutta niistä löytyi myös keskenään samoja lajeja. Lisäksi varpukasveilla (kanerva, mustikka ja puolukka) oli erilainen vaikutus ympäröivän maan kemialliseen koostumukseen kuin männyllä. Tämän laboratoriomittakaavan mikrokosmoskokeen tulosten perusteella kanervakasveilla on merkittävä vaikutus boreaalisen metsämaan prosesseihin ja mikrobien monimuotoisuuden ylläpitämiseen.

Kasvien maahan syöttämien hiiliyhdisteiden pääsyn rajoittaminen aiheutti muutoksia humusmaan sieniyhteisön rakenteeseen kolmivuotisen kenttäkokeen aikana. Lahottajasienten ja ektomykorritsasienten on arveltu kilpailevan maaperässä samoista

ravinteista. Jos kasvien hiilisyöte ektomykorritsasienille lakkaa, lahottajasienten on arveltu yleistyvän ja estävän ektomykorritsasienten kasvua. Tämän sieniryhmien välisen oletetun kilpailun taustalla olevaa mekanismia kutsutaan Gadgil-ilmiöksi. Tässä väitöskirjatyössä kuvatun kolmivuotisen kenttäkokeen tulokset eivät kuitenkaan tue Gadgil-ilmiötä vaan viittaavat siihen, että lahottajasienet ja ektomykorritsasienet suosivat erilaisia ravinteiden lähteitä ja eri ekolokeroita. Lisäksi tuloksien perusteella maaperän mikrobit ovat sopeutuvaisia ja kykenevät mukautumaan tilapäisiin häiriöihin.

Tässä väitöskirjatyössä saatiin uusia näkökulmia kasvin vaikutuksesta juuriston ja maaperän sieniyhteisön rakenteeseen ja toimintaan sekä maaperän kemialliseen koostumukseen. Tämän väitöskirjatyön tulokset viittaavat siihen, että tarvitsemme lisätietoa kasvien ja mikrobien vuorovaikutuksesta sekä näiden vuorovaikutusten merkityksestä maaperän prosesseille, jotta voimme ennustaa muuttuvan ilmaston vaikutusta boreaalisen metsämaan hiilenkierrossa.

Introduction

1.1 The boreal forest environment

Globally, forests cover approximately one third of the Earth´s land surface area (The World Bank, 2018). The forests are globally significant reservoirs of carbon (C) with over 45% of the terrestrial C stored in their vegetation and soils (IPCC, 2000). It is estimated that, globally, 42% of the forest C is in roots and other live biomass, 44% in soils, and the rest in litter and deadwood (Pan *et al.*, 2011). Approximately one third of the world´s forests are boreal forests (IPCC, 2000). From the total C stock of the Earth´s forests, approximately 32% is stored in the boreal forest ecosystems. Unlike in other forests, in boreal forests most of the C is stored in the soils (60%) and only 20% is in live biomass (Pan *et al.*, 2011). Because of the high C storage, the effects of plant-microbe interactions and changes in these interactions to the soil C cycling in boreal forest ecosystems are acquiring increasing interest.

1.1.1 The biome in boreal forests

The vegetation in boreal forests is commonly dominated by coniferous trees and the ground vegetation consisting typically of ericoid dwarf shrubs from *Ericaceae* family as well as herbs and various mosses (Kuuluvainen *et al.*, 2017). For example, in Finland, the most dominant tree species are Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*), and the most common ericoid dwarf shrubs are bilberry (*Vaccinium myrtillus*), lingonberry (*Vaccinium vitis-idaea*), crowberry (*Empetrum nigrum*) and heather (*Calluna vulgaris*) (Tomppo *et al.*, 2011). The boreal forest soils are typically podzolic (Blake *et al.*, 2008a; DeLuca & Boisvenue, 2012), where the upper most organic (O) soil horizon consists of fresh litter (O_L), fermenting litter (O_F) and humified organic matter (O_H) layers. The depth of the soil organic horizon in boreal forests varies highly from centimeter-scale up to one meter (Clemmensen *et al.*, 2013). Under the organic horizon lies the A horizon, where humified organic and mineral matter are mixed with each other, followed by the eluvial (E) horizon which is weathered by the leaching of the base cations to the underlying mineral B horizon (Blake *et al.*, 2008b; Gupta *et al.*, 2008). The organic horizons of the boreal forest soils contain more C stored per volume than other soil horizons (DeLuca & Boisvenue, 2012).

The boreal forest soils are typically considered to be nutrient poor soils since the nutrients are stored also in forms that are not easily available to the plants. To enhance their nutrient and water uptake, plants form symbioses with various fungi which are efficient in scavenging nutrients from complex organic matter (Smith & Read, 2008). In the soils, the plant roots and fungi interact also with other soil inhabiting organisms, such as bacteria and nematodes. The fungi, bacteria and nematodes participate in the nutrient cycling, and are all important in soil organic matter (SOM) formation and modification as well as nutrient scavenging. Typically, the amount of bacterial biomass is distributed

evenly along the organic horizon whereas the fungal biomass declines with the depth of the soil layer being highest in the fresh litter layers (Šnajdr *et al.*, 2008; Baldrian *et al.*, 2010). Moreover, the soil microbes can also act as a source of nutrients to the nematodes and other soil predators (Fitter & Garbaye, 1994).

1.1.2 Organic matter in boreal forest soils

In the boreal forest soils, C is stored in the SOM formed from partly decomposed plant litter, root fragments, plant exudates, soil animal litter and microbial biomass (Clemmensen *et al.*, 2013). Overall, the accumulation of SOM is considered to be caused by the slow decomposition rates and plant litter quality (Berg *et al.*, 2001; Wardle *et al.*, 2012). In boreal forest, the litter input is rich in phenolic compounds from plant roots as well as falling branches, needles and shoots from coniferous trees, and leaves and berries from shrubs. After partial degradation of this litter, the more difficultly degradable residuals, i.e. complex polymers such as phenols and humic compounds, build up in the SOM (Berg *et al.*, 2001; DeLuca & Boisvenue, 2012). These complex polymers are considered to be stable and have a long residence time in the soils (DeLuca & Aplet, 2008). Also the soil microbial necromass has an important role in forming the SOM, and can increase the soil stable C pool (Clemmensen *et al.*, 2015; Liang *et al.*, 2017). However, the estimation of the magnitude of the SOM accumulation by microbial necromass is challenging and it depends on multiple factors, such as the plant litter quality, intensity of the photosynthetic C flow from the plant, and chemical content of the fungal necromass (Clemmensen *et al.*, 2015; Fernandez *et al.*, 2016; Liang *et al.*, 2017).

Although the boreal forest soils have high nitrogen (N) content, they are considered to be limited by the amount of available N (Schulten & Schnitzer, 1998; Korhonen *et al.*, 2013). The plants can directly uptake the inorganic forms of N, such as ammonium (NH_4^+) and nitrate (NO_3^-), and organic N only in the form of amino acids (Näsholm *et al.*, 2009). From the N deposited in the soil organic layer, the majority is complex with soil particles, and even from the degradable N pool only a fraction is readily available for the plants as NH_4^+, NO_3^- or amino acids (Korhonen *et al.*, 2013). Typically the soil organic N, such as proteins, amino acids, chitin and polyamines, are complexed with phenolic plant secondary metabolites, such as tannins and terpenes (Adamczyk *et al.*, 2018). To obtain plant-utilizable N from these complexes, these bonds must be first broken.

Phosphorus (P) availability in the soils is also limited and only a fraction of it is soluble and available to the plant roots (Blake *et al.*, 2008c). In boreal forest soils, the majority of P is immobilized in inorganic or in organic forms which are inaccessible by plants (Bolan, 1991). Most of the organic and inorganic P is bound to soil particle surfaces and with soil minerals, or complexed with SOM (Bolan, 1991; Blake *et al.*, 2008c). The most common organic P forms present in the soils are nucleic acids, phospholipids and inositol phosphates, such as phytates, which are abundant in humic soils (Bolan, 1991). Before the plants can utilize the soil P, the P bound to soil particles has to be solubilized.

Typically, N and P are released from the complexes enzymatically. Mycorrhizal fungi are able to produce N uptake related enzymes, such as chitinases and proteases, as well as P uptake related phosphatase enzymes (Bolan, 1991; Kerley & Read, 1995, 1997; Blake *et al.*, 2008c; Heinonsalo *et al.*, 2015, 2017). However, soil tannins and terpenes can bind to the plant biomass degrading enzymes and increase or more commonly inhibit their activity (Baldrian, 2006; Adamczyk *et al.*, 2015, 2017). For the non-enzymatic release of nutrients, mycorrhizal fungi can produce organic acids, which react with the minerals to release the bound P or N compounds (Bolan, 1991).

1.2 Fungi in boreal forests

The fungi in boreal soils can be divided based on their lifestyle to three main ecophysiological guilds: mycorrhizal, saprotrophic or pathotrophic. However, in nature the lifestyle of the fungi can vary depending on their lifecycle or the surrounding environment, i.e. all the fungi associated with plant roots do not necessarily have a mycorrhizal lifestyle, and in some rare cases, saprotrophs can colonize the fine roots with no visible indications of disease (Vasiliauskas *et al.*, 2007). Nevertheless, all these fungi have their own important ecological roles in the environment, and this PhD thesis focuses on the ecological role of organic O_F and O_H horizons (later referred to as "humus") inhabiting plant root-associated fungal communities as well as saprotrophic and pathotrophic fungal communities.

1.2.1 Saprotrophic fungi

The saprotrophic and pathotrophic fungi maintain the cycle of nutrients by modifying the cell-wall structures of tree biomass and necromass, and making the nutrients more available for other microbes, animals and plants (Niemelä, 2006). Saprotrophic fungi obtain their nutrients by decomposing dead organic matter, and they break down the plant cell wall structures enzymatically by producing various organic matter degrading enzymes or chemically by Fenton chemistry (Hatakka & Hammel, 2010). The most efficient degraders of plant biomass are the wood-rotting basidiomycete fungi, which are further divided into white-rot and brown-rot fungi, depending on how they decay the wood. White-rot fungi have a wide repertoire of genes encoding various lignin and its subunits modifying oxidative enzymes as well as various cellulose and hemicelluloses degrading enzymes (Floudas *et al.*, 2012; Rytioja *et al.*, 2014). Brown-rot fungi are not in general able to modify lignin enzymatically but they utilize hydroxyl radicals produced by Fenton chemistry to expose the cellulose and hemicellulose chains, which can be further degraded with cellulose and hemicelluloses acting enzymes (Hammel *et al.*, 2002; Hatakka & Hammel, 2010).

The soil and litter inhabiting saprotrophs differ from the wood-decaying fungi in terms of their enzymatic machinery (Floudas *et al.*, 2012; Rytioja *et al.*, 2014; Kohler *et al.*, 2015) and in their capability to grow in the presence of humic substances (Steffen *et al.*, 2002; Heinonsalo *et al.*, 2012; Morin *et al.*, 2012). Furthermore, the soil and litter

saprotrophs are efficient degraders of SOM and can utilize the white-rot type of approach by producing lignin modifying oxidative enzymes as well as cellulose and hemicelluloses attacking enzymes (Steffen *et al.*, 2002; Morin *et al.*, 2012). In contrast to the wood-decaying saprotrophs, litter and soil saprotrophs contain a wider repertoire of genes encoding pectin modifying enzymes (Rytioja *et al.*, 2014). The litter and soil saprotrophs generally dominate the fresh litter layer of the boreal forest soils (Lindahl *et al.*, 2007; Santalahti *et al.*, 2016), where they break down the fallen litter with an initially high C/N ratio (Lindahl *et al.*, 2002; Saunders *et al.*, 2011). During the litter degradation, the saprotrophs utilize most of the easily available C from the litter resulting in a lower C/N ratio of the decomposed organic matter.

1.2.2 Plant root-associated fungi

Mycorrhizal fungi live in symbiosis with plants and form an interface with the plant root. This interface allows bilateral nutrient and energy transport between the plant and fungus (Smith & Read, 2008). These mycorrhizal structures between the fungus and the plant can be divided into two main categories: endo- and ectomycorrhizas (Brundrett, 2004). In endomycorrhiza, the fungal hyphae penetrate through the root-cell walls and lives between the cell walls and cell membranes. In ectomycorrhiza, the fungal hyphae grow on the root surface and between the root-cells without penetrating the root-cell walls. Generally, mycorrhizal fungi receive most of their energy through the plant-fungal interface as photosynthetically fixed C. In turn, mycorrhizal fungi provide their host plants better access to water and critical nutrients, such as N and P, as well as protect their host plant root-system against pathotrophic fungi (Smith & Read, 2008).

In the boreal forest ecosystem, the most common mycorrhizal fungi are ectomycorrhizal (ECM) fungi, which belong typically to the phylum Basidiomycota, and ericoid mycorrhizal (ERM) fungi, which are most commonly from the phylum Ascomycota. From these, the ECM fungi form ectomycorrhiza-structures with trees, while the ERM fungi form endomycorrhiza-structures with ericoid shrubs. Compared to the ERM fungi, ECM fungi are an evolutionarily older lineage (Brundrett, 2002), more widely studied, and many such fungi have a generally accepted ECM status (Tedersoo *et al.*, 2010). From an evolutionary perspective, the ERM fungal lineage is relatively young (Brundrett, 2002; Martino *et al.*, 2018), and so far few fungi have obtained ERM status from re-synthesis tests. The ascomycete fungi *Rhizoscyphus ericae* (synonym *Pezoloma ericae*, formerly *Hymenoscyphus ericae*)*, Meliniomyces variabilis,* and *Oidiodendron maius*, as well as basidiomycete fungi from clade B Sebacinales (syn. Serendipitaceae) have an established ERM status (Vrålstad *et al.*, 2000, 2002; Rice & Currah, 2006; Selosse *et al.*, 2007, 2009; Weiß *et al.*, 2011; Vohník *et al.*, 2012, 2016), and are commonly detected in the ericoid plant roots. From these *R. ericae* and *M. variabilis* are members of the *R. ericae* aggregate, which includes also *Meliniomyces bicolor*, *Meliniomyces vraolstadiae* and *Cadophora finlandica* (Vrålstad *et al.*, 2000; Hambleton & Sigler, 2005; Grelet *et al.*, 2009, 2010). From these, *M. bicolor* has both ECM and

ERM abilities (Villarreal-Ruiz *et al.*, 2004), but is suspected to have more affinity towards the ericoid plants (Kohout *et al.*, 2011).

Some plant root-associated fungi can live on the roots as endophytes or pathogens without forming the traditional interfaces that allow bilateral nutrient exchange with the plant. In fact, some of the fungi that form mycorrhizal associations with one plant species can live as endophytes with other plant species (Brundrett, 2004). This is also the case with the ERM fungus *M. variabilis*, which can inhabit conifer roots as an endophyte (Piercey *et al.*, 2002; Vohnik *et al.*, 2013). These endophytes can have positive, neutral or negative effect on the plants growth. For example, members of the endophytic *Phialocephala fortinii* s. l.–*Acephala applanata* species complex (PAC) are reported to be present in both ericoid and conifer roots (Jumpponen *et al.*, 1998; Menkis *et al.*, 2004; Grünig *et al.*, 2008; Grelet *et al.*, 2010; Bent *et al.*, 2011; Walker *et al.*, 2011; Toju *et al.*, 2016a; Bruzone *et al.*, 2017; Heinonsalo *et al.*, 2017), and appear to have varying ecological roles from mutualistic to saprotrophic (Haselwandter & Read, 1982; Jumpponen *et al.*, 1998; Jumpponen, 2001; Menkis *et al.*, 2004; Addy *et al.*, 2005; Lukešová *et al.*, 2015). Some are even found to enhance the plant growth when co-existing with mycorrhizal fungi (Reininger & Sieber, 2012, 2013).

1.2.3 Mycorrhizal fungi as decomposers

Recent genome sequencing projects have revealed that during the evolution of both the ECM and ERM lifestyle, these fungi have diverged several times from the saprotrophic lineage (Tedersoo *et al.*, 2010; Ryberg & Matheny, 2012; Martin *et al.*, 2016; Martino *et al.*, 2018) and have diverse saprotrophic ancestors. As a lineage the ECM fungi are older than ERM fungi, and the divergence of the ERM fungi from the saprotrophic lineage is speculated to be ongoing (Martino *et al.*, 2018).

During their separation from early saprotrophs, ECM fungi lost most of their ancestral saprotrophic apparatus and the capability to produce a wide range of organic matter degrading enzymes (Kohler *et al.*, 2015; Martin *et al.*, 2016; Martino *et al.*, 2018). However, some ECM can produce lignin modifying oxidative enzymes (Bödeker *et al.*, 2009, 2014; Heinonsalo *et al.*, 2012; Shah *et al.*, 2016) or degrade SOM by utilizing Fenton chemistry similar to brown-rot fungi (Rineau *et al.*, 2012). The ability of ECM fungi to degrade SOM varies greatly between the different ECM lineages (Pellitier & Zak, 2018) and they are generally less efficient degraders than saprotrophs (Tanesaka *et al.*, 1993). Since ECM fungi are supported by the photosynthates from their host plants, their saprotrophic activity is probably a side effect in the process of scavenging for nutrients from the soil (Lindahl & Tunlid, 2015). The released C can be exploited by microbes living in close vicinity of the ECM hyphae, or in cases when the C flow from the plant is insufficient, by the ECM fungi themselves (Talbot *et al.*, 2008).

Compared to the ECM fungi, the ERM fungi have a wider repertoire of organic matter degrading enzymes encoding genes (Kohler *et al.*, 2015; Martino *et al.*, 2018) and they are capable of degrading cellulose and other complex SOM compounds (Rice & Currah, 2001, 2005, 2006; Piercey *et al.*, 2002; Thormann *et al.*, 2002). Some ERM fungi are

considered to have a dual role in the soils; in addition to forming ERM associations with ericoid plants, they can live as saprotrophs, such as *O. maius*, or as endophytes in other plants roots, such as *M. variabilis*. So far, only four ERM fungi, *R. ericae*, *M. variabilis*, *M. bicolor* and *O maius*, have been whole genome sequenced (DOE JGI, accessed 20.02.2018). The genomic information of these ERM fungi is suggested to indicate that they have all adapted to different habitats (Martino *et al.*, 2018). For example *O. maius*, has preserved an extensive number of cellulose degrading genes (Kohler *et al.*, 2015) and is an efficient peat decomposer (Rice & Currah, 2001, 2002; Piercey *et al.*, 2002). The other three recently sequenced fungi, all members of the *R. ericae* aggregate, possess a wide repertoire of chitin degrading enzymes encoding genes (Martino *et al.*, 2018). In addition, *R. ericae* is able to acquire N from chitin containing substrates, such as fungal mycelia (Kerley & Read, 1995, 1997, 1998).

1.3 Fungal interactions in boreal forest soils

In the soils fungi, bacteria, nematodes and plant roots interact with each other, and can have positive, negative or neutral effect on each other's functionality. These interactions are important for ecosystem functioning, and various processes in boreal forest soils are interlinked with each other. This PhD thesis focuses on the interactions a where fungus is one of the interacting partners. These include interactions between fungi and bacteria, saprotrophic and mycorrhizal fungi, as well as mycorrhizal fungi and their host plants.

1.3.1 Interactions between fungi and bacteria in boreal forest

The boreal forest soils contain a wide range of bacterial species (Fierer *et al.*, 2007; Baldrian *et al.*, 2012). Among these, bacteria from the classes Acidobacteria, Alphaproteobacteria and Actinobacteria, are commonly detected in ericoid and pine dominated soils (Männistö *et al.*, 2007; Baldrian *et al.*, 2012; Timonen *et al.*, 2017). Bacteria can also participate in SOM degradation, but generally with lower rates (Baldrian, 2017a). Especially, species from Actinobacteria can contribute to SOM degradation by producing various lignin and cellulose degradation related enzymes (Schäfer *et al.*, 2010). In addition, bacteria of the order Rhizobiales, from class Alphaproteobacteria, are known to be able to participate in the N fixation in soils and plant roots (Jones, 2015). In addition to their N fixation ability, bacteria of the genus *Rhizomicrobium* can utilize plant cell wall sugars, such as cellobiose, xylose, galactose and arabinose, for energy (Kodama & Watanabe, 2011). The N fixing bacteria are proposed to supply N to the fungus in exchange for exudates from the fungi (de Boer *et al.*, 2005).

Soil inhabiting fungal and bacterial communities participate together in SOM and nutrient cycling by producing various organic matter degrading enzymes and providing nutrients to each other (de Boer *et al.*, 2005). In addition, fungi and their exudates provide different ecological niches for bacteria and may assist the growth of different bacteria by

transporting plant exudates or producing exudates, or repress bacterial growth by producing bactericides (de Boer *et al.*, 2005). Furthermore, the mycorrhizal fungi are important in shaping the associated bacterial community (Timonen *et al.*, 1998). Mycorrhizal roots harbour different bacterial communities than the surrounding soils (Skyring & Quadling, 1969; Timonen *et al.*, 2017), whereas the soil inhabiting bacterial community is mostly influenced by the soil chemistry and pH (Jeanbille *et al.*, 2016). In turn, the bacteria can stimulate the establishment of the mycorrhizal interface between the plant and fungus as well as promote the formation of fungal fruiting bodies (Garbaye, 1994; de Boer *et al.*, 2005). In addition, the soil bacteria are actively participating in degradation of dead fungal mycelia (Brabcová *et al.*, 2016; Baldrian, 2017a).

1.3.2 Antagonism between mycorrhizal and saprotrophic fungi

In the soils, ECM and saprotrophic fungi are found to interact and acquire nutrients from each other (Lindahl *et al.*, 1999, 2001; Leake *et al.*, 2001), but they tend to dominate different layers of the soils. The fresh litter layers are usually dominated by saprotrophic fungi whereas ECM fungi dominate the humus layers (O'Brien *et al.*, 2005; Lindahl *et al.*, 2007; Santalahti *et al.*, 2016), and the spatial separation of these two fungal guilds could be a result of antagonism between them. Based on the "Gadgil effect" theory, as the SOM in the humus layer is a more difficult substrate, the saprotrophs are outcompeted by the ECM fungi due to the secured C flow from the plants (Gadgil & Gadgil, 1971, 1975). This phenomenon is called the "Gadgil effect" and competition for N and P might be one of the mechanisms explaining this theory (Fernandez & Kennedy, 2016), since substantial availability of N is critical to the degradative activity of saprotrophs (Boyle, 1998; Boberg *et al.*, 2008, 2011). As ECM and ERM fungi are efficient in scavenging and utilizing the N from complex matter (Rineau *et al.*, 2012; Lindahl & Tunlid, 2015), their activity can cause the substrate to become more limited by the available N and thus inhibit the growth of soil and litter saprotrophs on that substrate.

Studies investigating the appearance and magnitude of the "Gadgil effect" have obtained contradictory results. Some studies have confirmed the presence of the "Gadgil effect" (Gadgil & Gadgil, 1971, 1975; Koide & Wu, 2003), some have observed no competitive interaction between ECM and saprotrophs (Mayor & Henkel, 2006; McGuire *et al.*, 2010), and some have even observed ECM fungi to have a positive effect on litter decomposition (Zhu & Ehrenfeld, 1996). Despite the controversial findings on the "Gadgil effect" magnitude, there is evidence of competition for nutrients between saprotrophic and ECM fungi. In certain conditions, *P. sylvestris* root colonizing ECM fungi are found to be able to capture P from saprotrophic mycelia (Lindahl *et al.*, 1999). However, this nutrient capture ability of ECM fungi from saprotrophs is dependent on the competitive strength of the saprotrophic fungi, i.e. when the saprotrophic fungus is grown on a substrate with easily utilizable C, such as wood blocks, it is able to capture P and C from the ECM fungus (Leake *et al.*, 2001; Lindahl *et al.*, 2001). The spatial separation of these two fungal guilds is proposed to also be driven by differences in their substrate preferences and nutrient acquisition abilities. As the saprotrophic machineries

of soil and litter saprotrophs and ECM fungi differ from each other based on the potential functionality (Kohler *et al.*, 2015), it may reflect their different niche preferences. In addition, recalcitrant SOM is proposed to be an unbeneficial substrate for the saprotrophs since the production of SOM degrading enzymes would consume more energy than they would obtain from the degraded substrate (Baldrian, 2009). Thus, the spatial separation of the ECM and saprotrophs could be a result of preference for different ecological niches rather than competition between these two guilds (Fernandez & Kennedy, 2016).

1.3.3 Interaction between ericoid and ectomycorrhizal plants and their associated fungi in the boreal forest ecosystem

To ensure their water and nutrient uptake in different environmental conditions and during different disturbances, plants can form associations simultaneously with different fungal species, and several root-associated fungi are found to co-exist and possibly compete in the plant roots (Kennedy, 2010; Bent *et al.*, 2011; Toju *et al.*, 2016b,a). All the plant root-associated fungi do not inevitably form mycorrhizal associations and some of them can live in the plant root-system as endophytes or pathotrophs. Plants can provide the root-associated fungi with different ecological niches, and different fungal species are typically specialized in forming mycorrhizal associations with certain plant species (Brundrett, 2002; Drenovsky *et al.*, 2004; Bougoure *et al.*, 2007; Ishida & Nordin, 2010; Tedersoo *et al.*, 2013). The host preference of root-associated fungi still varies highly between fungal species (Molina *et al.*, 1992; Bougoure *et al.*, 2007; Ishida & Nordin, 2010; Toju *et al.*, 2016a), and some studies indicate that regionality rather than the identity of the host species is the dominating factor determining the plant root-associated fungal community (Kjøller *et al.*, 2010; Walker *et al.*, 2011).

Plant root-associated fungal communities are proposed to participate in common mycorrhizal networks (CMN) where the roots of at least two plants are linked together through fungal mycelia (Selosse *et al.*, 2006). The CMN can be formed by one individual fungal species colonizing the roots of at least two different individual plants, or by two individual fungi of the same or genetically closely related species colonizing the different individual plant roots (Selosse *et al.*, 2006). Through CMN plants can interact as well as exchange carbon and nutrients with each other (Simard *et al.*, 1997; Simard, 2012). This network can give the plant and fungi competitive advantages in different environmental conditions, such as during drought or under lowered photosynthesis rates (Simard *et al.*, 1997; Selosse *et al.*, 2006; Simard, 2012). Since the members of the *R. ericae* aggregate can inhabit the roots of both ericoid and coniferous plants, the members of the *R. ericae* aggregate are proposed to participate to formation of the CMN in the boreal forest ecosystem (Vrålstad, 2004; Bent *et al.*, 2011).

Understanding the variety of plant roots and rhizosphere associated fungi is important when predicting the below-ground functional responses to the above-ground changes. The anthropogenic CO_2 levels in the atmosphere are estimated to rise and the climate to warm during the next decades (Collins *et al.*, 2013). This will lead to prolonged growing

seasons in the boreal zones and can increase photosynthetic C input from the plants to the soil and plant root microbes (Sevanto & Dickman, 2015). The increased C input from the plants can accelerate the activity of the soil microbial community, production of the organic matter degrading enzymes, and degradation of the older and more recalcitrant SOM (Kuzyakov *et al.*, 2000; Fontaine *et al.*, 2007; Kuzyakov & Blagodatskaya, 2015). This phenomenon where the increased input of easily decomposable C changes the recalcitrant SOM degradation patterns is called the "priming effect". The mechanisms behind the "priming effect" have received increasing interest as the soil microbial communities' responses to the changing climate are studied. Although the plant-derived C is an important factor when determining the soil inhabiting microbial community´s activity, recently it has been shown that also other factors, such as N availability and C/N ratio, have an important effect on the strength of the "priming effect" (Blagodatskaya & Kuzyakov, 2008; Chen *et al.*, 2014; Luo *et al.*, 2016).

2 Aims of the study

The main objectives of this PhD research work were to investigate the effect of the plant-derived photosynthetic carbon on the fungal community structures and soil organic matter decomposition in both a laboratory-scale microcosm study as well as a field experiment. In addition, the aim was to identify photosynthates directly from the plant utilizing root-associated fungi by using a DNA-based ^{13}C stable isotope probing technique.

Plant-fungal interactions are important for the whole boreal forest ecosystem. However, the host preference of ericoid root associated fungi is not yet sufficiently understood. In addition, the changes to soil chemical composition driven by the different boreal forest plants and their fungal associates is not yet fully described. The microcosm study described in publications **I** and **II** aimed to provide further information to these questions.

In the boreal forest ecosystem, mycorrhizal fungi provide host plants with better access to water and essential nutrients. In return they obtain most of their energy in the form of sugars from the plants. Due to the secured photosynthetic flow from the plants, mycorrhizal fungi are suggested to dominate the humus layer and outcompete the saprotrophs. This antagonism is suggested to result in the spatial separation of mycorrhiza to the humus layer and saprotrophs to the litter layer. However, the responses of the soil microbial community to the long-term restriction of the photosynthetic carbon flow from plant to soil remains to be elucidated. This question was examined in the field experiment described in manuscript **III**.

The specific aims of this work were:

- to study the individual effects of the common boreal forest plants, heather (*Calluna vulgaris*), bilberry (*Vaccinium myrtillus*), lingonberry (*Vaccinium vitis-idaea*) and Scots pine (*Pinus sylvestris*), on the soil chemical composition, fungal community structure, and microbial SOM degrading enzymatic activities (**I**, **II**).
- to identify those root-associated fungi which utilize photosynthetic C from Scots pine and three ericoid plants (*C. vulgaris*, *V. myrtillus*, and *V. vitis-idaea*) (**I**).
- to study how the restriction of direct carbon flow from the plants affects the soil microbial community structure and dynamics (**III**).

3 Summary of materials and methods

The experimental setups and materials and methods are described in detailed in publications and manuscript (**I**–**III**), and all methods used are summarized here in Table 1. The principles of the experimental setups as well as the key methods used in this work are described here in this section.

Table 1. List of all the methods used in the publications and manuscript **I–III**.

Method	Publication
Experiment	
Laboratory scale microcosm experiment	I, II
Field experiment	III
Soil chemistry	
pH	II
Total C, N and SOM content	II
Ammonium-N, nitrate-N and total free amino acid content	II
Recalcitrant and degradable N pools	II
Condensed tannins and total water-soluble phenolic compounds	II
Amount of enchytraeid worms	II
Fourier transform infrared spectroscopy measurements	II
Functionality of the microbial community	
Extraction of extracellular enzymes from soil with filter centrifugation	II
Enzyme activity measurements with fluorometric assay	
Acid phosphatase (EC 3.1.3.2)	II
Chitinase (EC 3.2.1.14)	II
β-glucosidase (EC 3.2.1.21)	II
β-glucuronidase (EC 3.2.1.31)	II
β-xylosidase (EC 3.2.1.37)	II
Cellobiohydrolase I (EC 3.2.1.91)	II
Leucine aminopeptidase (EC 3.4.11.1)	II
Potential microbial community	
Microscopy of ericoid roots	I
DNA extraction	
CTAB-based method for root samples	I
Commercially available extraction kit based method (Macherey-Nagel) for soil samples	I, III
Stable isotope probing	
$^{13}CO_2$-labeling	I
Density gradient ultracentrifugation	I
Quantitative PCR (qPCR)	I
GeoChip 5.0S	III
MiSeq sequencing	
Fungal ITS2 region	I, III
Bacterial 16S rDNA region	III
Processing and analysing of gene sequence data	
With mothur in CSC environment	I, III
With R program	I, III
Statistical analysis	
With R program	I, II, III
With SPSS	II

3.1 Experimental setup

The soil for both the laboratory scale microcosm study (publications **I**, **II**) as well as for the mesh bags in the field experiment (manuscript **III)** was collected from the organic O_F and O_H layers (referred here as "humus") from a forest surrounding the Hyytiälä field station SMEAR II (Station for Measuring Forest Ecosystem-Atmosphere Relations) in Southern Finland (61°51' N, 24°17' E) (Hari & Kulmala, 2005). There, Scots pine (*P. sylvestris* L.) is the dominating tree species and the ground vegetation is dominated by shrubs (*C. vulgaris*, *V. vitis-idaea* and *V. myrtillus*) and mosses (*Dicranum polysetum*, *Hylocomium splendens* and *Pleurozium schreberi*) (Kolari *et al.*, 2006). The collected humus was homogenized and sieved (4 mm mesh) but left otherwise untreated. The humus served later in the experiments also as a repository for the fungal and bacterial inocula.

3.1.1 Microcosm experiment

For the microcosm experiment (publications **I**, **II**), 14 replicate microcosms were constructed for five treatments containing the ericoid plants, heather (*C. vulgaris* (L.) Hull), bilberry (*V. myrtillus* L.), lingonberry (*V. vitis-idaea* L.), the ectomycorrhizal Scots pine (*P. sylvestris* L.) as a control species, and unplanted controls containing only soil. For investigating the roots and rhizosphere associated microbial community´s ability to obtain organic-N, necromass of fungus grown on ^{15}N-label containing media was placed in mesh bags (pore size 50 µm) into half of the microcosms during their construction (publication **II**). The microcosms (Perspex®, 20x30 cm, soil thickness 4 mm) were placed vertically and the roots were protected from the light. The seedlings were grown under average forest floor light intensity at temperatures of 18 °C and 14°C during an 18 hour-long day and 6 hour-long night, respectively. During the experiment, the soil moisture was kept stable by watering with de-ionized and filtered (0.22 µm) water. The studied plant species had different growth rates (Kulmala *et al.*, 2017) and in order to adjust for this, *C. vulgaris* was grown for 547 days, *V. myrtillus* for 540 days, *V. vitis-idaea* for 582 days, and *P. sylvestris* for 412 days, resulting in approximately the same sized seedlings at the time of the harvest (Figure 1).

Before harvest, six replicate plants from each treatment were exposed to $^{13}CO_2$ for two hours per day over five consecutive days. At the harvest, visible fine roots were picked away from the soil samples, and soil particles were washed away from root samples. A simplified procedure describing the aspects studied from each replicate plant is shown in Figure 2. For soil chemistry and enzyme activity analyses (publication **II**), soil from all 14 replicates from the five different treatments were used. For DNA-based fungal community analyses (publication **I**), six replicate unplanted microcosms and six replicate plants from each species exposed to $^{13}CO_2$ were processed.

Figure 1. Example of microcosms of each the studied plant species (*C. vulgaris*, *V. myrtillus*, *V. vitis-idaea* and *P. sylvestris*) at the time of the harvest. (Photos: Jussi Heinonsalo)

3.1.2 Field experiment

The field experiment was conducted at Hyytiälä forestry field station (manuscript **III**). To discriminate the photosynthetic C flows, mesh bags with three different pore sizes (1000 µm, 50 µm and 1 µm (Figure 3)) were filled with the sieved and homogenized fresh humus corresponding to 14.2 g of dry weight. The bags were buried between organic and mineral soil horizons in three different sampling areas over 50 m apart from each other. These different mesh sizes allowed penetration of both fungal hyphae and fine roots (1000 µm), allowed fungal hyphae to penetrate but restricted plant roots (50 µm), or prevented the entrance of both fungal hyphae and plant roots (1 µm) (Wallander *et al.*, 2001). One set of bags (n=18 for each mesh treatment) was harvested yearly in late September after first, second and third growing seasons, resulting in a total of 162 samples.

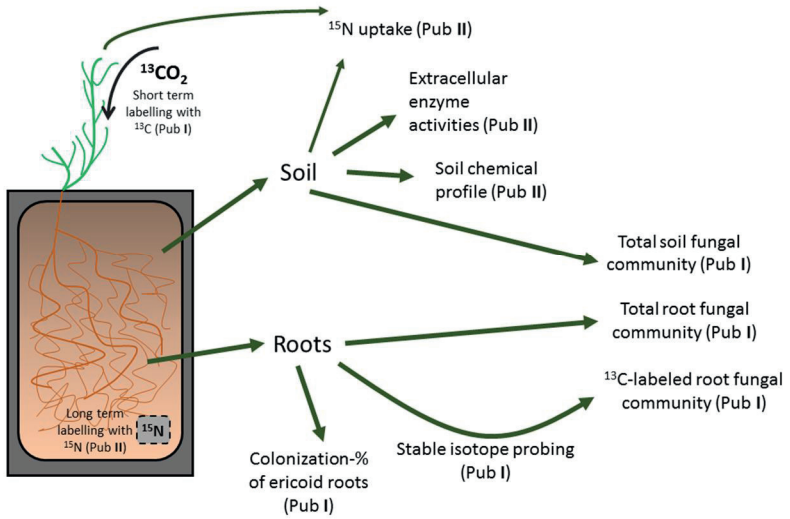

Figure 2. Simplified schematic representation of different aspects studied in the laboratory-scale microcosm experiment in publications **I** and **II**. For publication **I**, the number of replicates is six for each plant species and unplanted microcosms. For publication **II**, 14 replicates from each plant species and unplanted microcosms were processed.

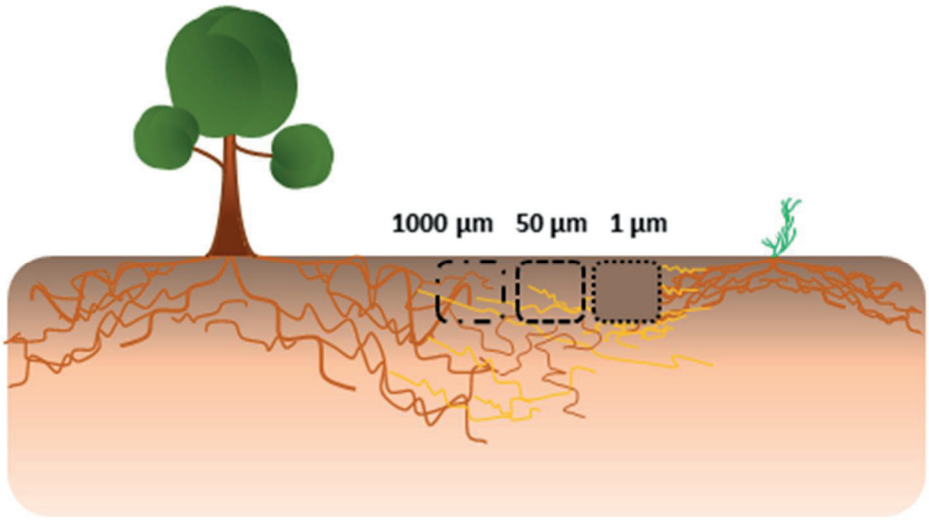

Figure 3. Schematic principle of the field experiment conducted in manuscript **III**. One set of bags was harvested yearly after first, second and third growing seasons. For each mesh size within each growing season, 18 replicate bags were collected, resulting in a total of 162 samples.

3.2 Extraction of extracellular enzymes and measuring enzyme activities from soils

In publication **II**, the extracellular enzymes were extracted from soil samples with a filter centrifugation method using Costar® Spin-X® centrifuge tubes with 0.22 µm nylon filters (Corning) as described in Heinonsalo *et al.* (2012). Briefly, triplicate 0.1 g (fw) samples of fresh humus were placed in Spin-X® filter centrifuge tubes and 100 µl of sterilized distilled water was added. The samples were incubated with water in +4 °C for 1 hour and then the extract was centrifuged through the filter with 161 000 × g for 30 min in +4 °C. The soil extracts were frozen until the time of enzyme activity measurements, at which point the soil extracts were defrosted at +4 °C, the triplicate subsamples were pooled together (V ≈ 300 µl) and adjusted to a final volume of 3 ml.

The activities of acid phosphatase (EC 3.1.3.2), cellobiohydrolase (EC 3.2.1.91), chitinase (EC 3.2.1.14), β-glucosidase (EC 3.2.1.21), β-glucuronidase (EC 3.2.1.31), β-xylosidase (EC 3.2.1.37), and leucine aminopeptidase (EC 3.4.11.1) were measured with a fluorometric assay. The incubation times were as described in Pritsch *et al.* (2011) and the reactions were carried out at +22 °C in pH 4.5, or pH 6.5 for leucine aminopeptidase (Courty *et al.*, 2005). The fluorescence was measured with a Victor3 plate reader (Perkin Elmer, Inc., USA) using wavelengths 355 nm for excitation and 460 nm for emission. The fluorescence reads from the samples were compared against standard curves prepared from 4-methylumbelliferone, or aminomethylcoumarin in case of leucine aminopeptidase. For determining the possible autofluorescence or auto-inhibition of fluorescence of the individual samples, 1 ml of each sample was heat inactivated at +85 °C for 2 hours to eliminate enzyme activity and cooled to +22 °C before performing the fluorometric assay similarly as with the actual samples. All the used substrates and standards were purchased from Sigma-Aldrich (USA).

3.3 Molecular biological methods

3.3.1 DNA extraction

In publication **I**, the fine roots were lysed with glass beads and the DNA was extracted from the lysed roots with a N-cetyl-N,N,N-trimethylammonium bromide (CTAB, Sigma) -based method (Timonen *et al.*, 2017). In publication **I** and manuscript **III**, the soil samples were homogenized and pre-lysed with ceramic bead tubes and DNA was extracted with the NucleoSpin® Soil DNA extraction kit (Macherey-Nagel GmbH&Co). All the extracted DNA samples were further purified with PowerClean® Pro DNA Clean Up kit (MO BIO Laboratories, USA). Concentrations of the purified double-stranded DNA samples were determined with a Qubit® 2.0 Fluorometer (Life Technologies, USA).

3.3.2 DNA-based stable isotope probing

From the extracted root-DNA, part of the extracted DNA was subjected to density gradient ultracentrifugation (publication **I**). The aim of this DNA-based ^{13}C stable isotope probing (^{13}C-DNA-SIP) method was to separate the assimilated ^{13}C from the plant utilizing root-associated fungi from the total root-associated fungal community.

For separating the ^{13}C-DNA-pool from the ^{12}C-DNA-pool, 1 g of CsCl was added into 1 ml of root-DNA. In addition, for lowering the buoyant density of the samples, 1 mg of ethidium bromide was added, after which the volume was adjusted to 6 ml (density: 1.59 mg ml^{-1}; refractory index: 1.3895, Atago, PAL-RI) and the solution was added to Sorvall 6 ml crimp tubes (Sorvall). For positive controls, ^{12}C- and ^{13}C-labeled DNA of *Methylocapsa acidiphila* were added to each density gradient centrifugation in separate tubes. After centrifuging the tubes at 48 000 rpm for 48 h at +20°C with a TV-1665 rotor (Sorvall), each of the Sorvall 6 ml crimp tubes were collected into ~150 µl fractions. The ethidium bromide was extracted from the samples with 1 vol of water saturated isobutanol (Merck, Germany), precipitated with ethanol using glycogen as carrier, and eluted to 50 µl of TE-buffer.

The purified fractions were screened with a Qubit 2.0 Fluorometer for DNA concentration assay and quantitative PCR (qPCR) with fungal 18S rDNA (ribosomal DNA) targeting FF390 and FR1 primers (Vainio & Hantula, 2000). Based on the qPCR and DNA concentration assays, the ^{13}C-DNA was determined to be in fractions collected from densities 1.59–1.61 g/ml (see Fig S3 in publication **I**).

3.3.3 MiSeq-sequencing

The fungal ITS2 (publication **I** and manuscript **III**) and bacterial V3–V4 regions of 16S rDNA (manuscript **III**) were sequenced from the root and soil samples using Illumina® MiSeq at the Institute of Biotechnology, University of Helsinki. For determining the total soil and root microbial community, 200 ng of the extracted DNA with equal concentrations was used (publication **I**, manuscript **III**). For the ^{13}C-labeled root fungal community analysis, equal volumes of the fractions with ^{13}C-DNA were pooled from each replicate plant for the MiSeq-sequencing.

Prior to sequencing, the target-DNA was amplified in a nested-PCR at the Institute of Biotechnology. In addition, the raw ITS2 (publication **I** and manuscript **III**) and 16S rDNA (manuscript **III**) sequences were pre-processed at the Institute of Biotechnology: the general read quality was checked with FastQC software (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and adapter and barcode sequences were trimmed with Cutadapt software (Martin, 2011).

3.3.4 GeoChip 5.0S microarray

The potential functional gene pools of the soil microbial community were investigated witha DNA-based GeoChip 5.0S (60K) microarray at the Glomics Inc. (USA) (manuscript **III**). For this, 1000 ng of DNA from six replicate samples from each

sampling area in each mesh treatment from each sampling year were pooled together. Here, n=3 for each mesh treatment in each growing seasons resulting in 27 samples.

The GeoChip 5.0S contains probes covering the C, N, P, and sulfite cycling related microbial genes, and also some contaminant degradation related genes (Van Nostrand *et al.*, 2016). At Glomics, the genomic DNA was mixed with random primers, labelled, purified, dried, and hybridized on the GeoChip 5.0S microarray (Van Nostrand *et al.*, 2016). The microarray signal intensities were pre-processed at Glomics Inc.

3.4 Processing and analyzing the MiSeq-sequencing data

The raw nucleotide sequences were deposited in the NCBI-database under Bioprojects PRJNA325435 (publication **I**) and PRJNA454770 (manuscript **III**). The sequence data were further filtered and clustered to operational taxonomic units (OTUs) using mothur (Schloss *et al.*, 2009). For identification, fungal sequences were aligned against UNITE-database (publication **I** and manuscript **III**) and bacterial sequences were aligned against mothur-formatted SILVA-databases. In manuscript **III**, the sequence data were further de-noised following the pipeline suggested by Callahan *et al.* (2016) using R program (R Core Team, 2016). The obtained fungal taxa were assigned in R program with metagMisc package (Mikryukov, 2017) to functional guilds according to the FUNGuild database (Nguyen *et al.*, 2016).

3.5 Statistical analysis of the data

For the sequence-based community data in publication **I** and manuscript **III**, the statistical and descriptive analyses were completed in the R programming environment (R Core Team, 2016). The level of statistical significance in all the analyses was set to *p*-value 0.05. The statistical significant differences in diversity indices in publication **I** and manuscript **III** were tested with non-parametric Kruskal-Wallis test using stats-package (R Core Team, 2016). The effects of plants and sample types (root vs. soil) in publication **I** or different mesh treatments in manuscript **III** on the microbial communities was tested from sequence counts normalized with library sizes using multivariate analysis of variance (MANOVA) from vegan-package (Oksanen *et al.*, 2017).

In publication **I**, Venn diagrams visualizing the number of shared and unique OTUs were constructed from the presence/absence transformed OTU data without singletons with gplots (Warnes *et al.*, 2016). The variance of fungal community structures between different samples in publication **I** were analyzed using a dissimilarity matrix calculated from the normalized sequence counts with vegan-package (Oksanen *et al.*, 2017) and visualized in principal coordinate analysis (PCoA) with labdsv-package (Roberts, 2016). In manuscript **III,** the fungal and bacterial orders, genera and species, as well as fungal functional guilds responding significantly to the mesh treatments were identified with DESeq2 package (Love *et al.*, 2014), where the level of significant log2 fold change value

was set to ±1 and adjusted p-value to 0.05. The log2 changes in DESeq2 were converted to fold changes by calculating 2 to the power of log2-value (i.e. $2^{\log2\ change}$).

In publication **II**, the canonical correspondence analysis (CCA) for the soil´s chemistry and enzyme activity data was performed with vegan-package with plant-species as a covariate. The grouping of the different plant types was visualized with ellipses representing the 60% confidence area of the observations and the significance of the CCA-axes and the covariate was evaluated with vegan-package.

In manuscript **III**, the gene diversities from GeoChip 5.0S were calculated from the probe-level data with the vegan-package. The signal intensities from different probes in the same gene family were combined and clustered into different functional subcategories based on the categories preset by Glomics. The statistical differences in the diversity indices and in the summed signal intensities from the GeoChip within each gene family in each sample were determined with the non-parametric Dunn´s test (Dinno, 2017).

3.5.1 Statistical analyses used in the summary of this PhD thesis

The figures 4 and 5, presented in the summary of this PhD thesis, were constructed in the R programming environment. For figure 4, the variance of only ^{13}C-utilizing fungal community structures between different plant species from publication **I** were analyzed with dissimilarity matrix from vegan-package and with PCoA from labdsv-package using the sequence counts normalized with library sizes. The species scores of the 10 most abundant ^{13}C-OTUs were extracted from the dissimilarity matrix with wascores-function from the vegan-package and visualized with the PCoA.

In figure 5, the soil chemical profiles and enzymatic activities from publication **II** were set as response variables in the CCA. The explanatory variables were selected among the 30 most abundant soil fungi from publication **I** with a model-build using ordistep-function from vegan-package. In the model build, the variable was kept in the model if its *p*-value was below 0.09. The explanatory variables selected for the model did not cross-correlate with each other. The statistical significance of the individual axes and the explanatory variables were determined with the anova.cca-function.

4 Main results and discussion

4.1 Individual effects caused by the common boreal forest plants

The individual effects of the boreal forest plants on the soil and root associated microbial community structure and activity, as well as the soil chemical profile, is not yet fully known. This PhD study aimed to elaborate the belowground changes driven by the three ericoid plants, *C. vulgaris*, *V. myrtillus* and *V. vitis-idaea*, as well as the coniferous tree *P. sylvestris* (publications **I** and **II**). In the publications **I** and **II**, all the studied ericoid plants were grown separately on originally the same organic soil containing a similar initial microbial inoculum. Overall, the ericoid plants shaped the fungal communities in their soils and roots and affected the soil chemical structure differently than *P. sylvestris*.

4.1.1 Differences in the fungal community structures between the roots and soils of different plant species

All the ERM plants, *C. vulgaris*, *V. myrtillus* and *V. vitis-idaea*, harbored in their roots different fungal communities than the ECM plant *P. sylvestris* (Figure 4 and Table 3 in publication **I**). The ericoid roots were dominated by the ERM fungi *Rhizoscyphus* and *O. maius*, which also utilized the photosynthetic-^{13}C from the ericoid plants. As expected, the ECM *Piloderma sphaerosporum* was dominant in *P. sylvestris* roots and soils, and utilized the photosynthetic-^{13}C from *P. sylvestris*. In addition, ERM *R. ericae* (OTU 1), ERM *M. variabilis* (OTU 4) and endophyte PAC aggregate member (OTU 10), utilized photosynthetic-^{13}C from all the studied plants (Table 2, publication **I**). However, their abundances in the ^{13}C-labeled root community differed depending on the plant species (Figure 4, Table 2).

The soil fungal community differed from the root-associated fungal community in all the studied plants (publication **I**). In addition, the studied plants caused more intensive changes to the root-associated fungal community than to the surrounding soils (Figure 4 in publication **I**). In publication **I**, the unplanted control as well as the planted soils were mostly dominated by fungi known to live as saprotrophs or to be able to adapt to the saprotrophic lifestyle if the photosynthetic C flow from the plant is restricted (Cannon & Kirk, 2007). Similarly in a field experiment, the soil associated fungal community has been detected to be more rich and diverse than the root-associated fungal community (Jumpponen & Johnson, 2005). According to previous observations from the same microcosm experiment as in publication **I**, the three ericoid plants and *P. sylvestris* induced more drastic plant-species-specific changes to the bacterial communities living in close vicinity to the roots than surrounding soils (Timonen *et al.*, 2017). Timonen *et al.* (2017) suggested that the plant photosynthetic C flow may enhance the activity of the root associated microbes and thus the plant identity causes more intensive changes to the

plant root-associated bacterial community than to the free-living bacterial community. The results from the fungal community are in line with this hypothesis, and the soil associated fungi might act as generalists inhabiting their own micro-niches (publication **I**).

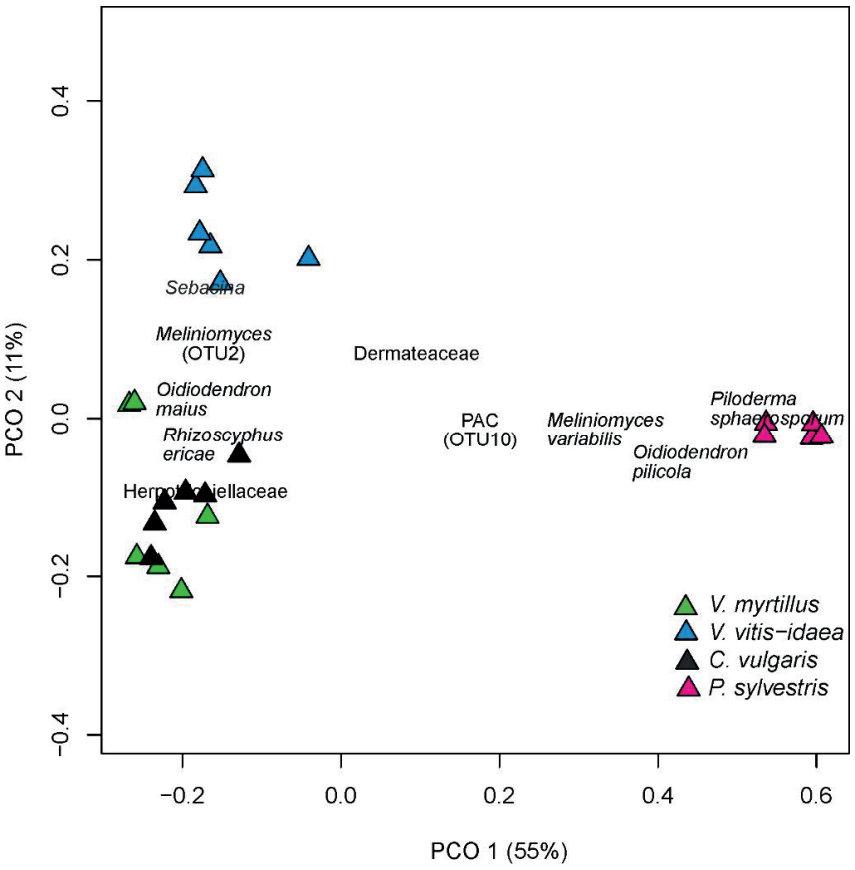

Figure 4. Principal coordinate (PCO) analysis from normalized fungal ITS2 gene sequence counts visualizing differences between the ^{13}C-labeled root communities of *C. vulgaris*, *V. myrtillus*, *V. vitis-idaea* and *P. sylvestris* described in publication **I**. The species-scores of 10 most abundant OTUs were extracted with wascores-function and shown in the figure.

Table 2. The relative abundances (% of the total sequence count per sample) and updated SH numbers (UNITE version 7.2) of the 30 most abundant operational taxonomic units (OTUs) in each sample type from publication **I** (^{13}C-labeled as well as total root and soil community).

OTU number	Identification	SH number	*V. myrtillus* Root			*V. vitis-idaea* Root			*C. vulgaris* Root			*P. sylvestris* Root			Control
			^{13}C-labeled community	Total community	Soil	^{13}C-labeled community	Total community	Soil	^{13}C-labeled community	Total community	Soil	^{13}C-labeled community	Total community	Soil	Soil
1	***Rhizoscyphus (Pezoloma) ericae***	SH214266.07FU	32.2	36.1	18.2	20.0	16.9	6.0	35.3	36.9	19.2	2.6	1.6	3.3	3.4
2	*Meliniomyces sp*	SH290729.07FU	8.7	13.1	8.1	15.2	13.1	11.8	4.0	6.9	6.4	0.6	5.5	7.6	7.3
3	*Oidiodendron maius*	SH216987.07FU	10.6	12.3	2.2	15.2	12.4	0.8	23.1	21.5	8.2	0.5	1.6	0.7	0.9
4	***Meliniomyces variabilis***	SH181078.07FU	1.8	2.8	2.2	5.2	5.3	2.8	5.5	5.5	2.5	24.6	20.6	3.4	5.9
5	*Sebacina*	SH197469.07FU	0.4	0.8	1.3	11.0	14.5	10.9	2.2	1.6	0.6	< 0.05	0.01	0.01	0.02
6	*Oidiodendron pilicola*	SH216991.07FU	< 0.05	0.02	5.2	< 0.05	0.1	9.7	0.1	0.1	4.4	0.4	0.8	8.3	11.4
7	*Dermateaceae*	SH215664.06FU	nd	0.01	5.9	0.1	0.03	8.8	< 0.05	0.1	4.2	0.1	0.4	9.3	10.6
8	*Herpotrichiellaceae*	SH226821.06FU	11.8	4.6	3.9	2.7	2.2	1.2	10.3	6.9	4.6	0.1	0.1	1.9	2.2
9	*Piloderma sphaerosporum*	SH196824.07FU	0.1	0.01	0.02	< 0.05	0.01	0.05	< 0.05	0.01	0.04	21.1	22.1	10.5	0.1
10	***Phialocephala fortinii* (PAC)**	SH213468.06FU	2.0	2.3	1.7	2.1	4.4	2.0	1.2	1.8	1.9	5.1	9.8	3.0	3.5
11	Lecideales	na	3.2	2.5	4.0	0.3	0.4	0.6	5.5	4.1	8.1	< 0.05	0.03	0.2	0.2
12	*Rhizoscyphus (Pezoloma) ericae*	SH181092.07FU	8.2	7.7	2.2	3.2	4.1	0.1	0.7	1.1	0.6	< 0.05	0.1	0.1	0.2
13	*Rhizoscyphus sp*	SH181092.07FU	3.6	3.3	1.1	4.5	4.5	0.5	1.9	1.8	1.8	< 0.05	0.1	0.3	0.5
14	Helotiales	SH020085.07FU	4.6	5.0	7.2	0.1	0.2	0.2	1.0	1.2	2.5	< 0.05	0.01	0.2	0.3
15	*Meliniomyces bicolor*	SH181080.07FU	nd	0.01	0.8	nd	0.03	0.5	nd	0.04	0.4	nd	4.3	7.6	1.2
16	*Sebacinales Group B sp*	SH203720.06FU	< 0.05	0.01	0.1	3.4	6.9	4.0	< 0.05	< 0.01	0.1	0.1	0.01	0.01	< 0.01
17	*Cenococcum geophilum*	SH199612.07FU	0.1	0.1	0.4	0.2	0.1	0.3	0.3	0.1	1.1	6.9	6.8	0.8	1.1
18	*Oidiodendron echinulatum*	SH216993.07FU	< 0.05	< 0.01	1.4	nd	0.02	2.2	nd	0.02	1.1	0.1	0.3	2.5	2.4
19	*Parmelia sp Hur R0090039*	SH197705.06FU	1.4	1.3	1.0	0.4	0.3	0.4	1.0	1.4	1.4	0.1	0.1	0.7	0.9
20	*Helotiaceae*	SH218283.07FU	1.5	0.7	1.6	0.6	0.3	0.3	1.9	1.6	3.1	0.2	0.01	0.1	0.2
21	*Hyaloscyphaceae*	SH496541.07FU	nd	< 0.01	1.1	nd	0.01	1.8	nd	0.02	0.9	nd	0.1	1.6	2.7
22	*Chloridium*	SH181582.07FU	< 0.05	0.03	0.8	nd	0.04	1.2	< 0.05	0.02	1.0	0.1	0.6	2.2	1.6
23	*Herpotrichiellaceae*	SH226825.06FU	nd	0.5	0.7	nd	0.1	0.5	nd	1.6	1.4	nd	0.03	0.3	0.3
24	*Meliniomyces vraolstadiae*	SH181085.07FU	< 0.05	0.02	0.2	0.2	0.1	0.5	0.1	0.04	0.1	7.3	3.5	0.4	0.3
25	Helotiales	SH218292.07FU	1.1	0.7	1.1	1.3	1.0	0.5	0.3	0.3	0.3	nd	0.01	0.2	0.1
26	*Hydnotrya michaelis*	SH221898.07FU	< 0.05	< 0.01	< 0.01	< 0.05	< 0.01	< 0.01	< 0.05	< 0.01	< 0.01	1.4	4.6	0.7	< 0.01
27	*Umbelopsis sp*	SH196092.07FU	nd	< 0.01	0.7	nd	0.01	1.0	< 0.05	0.01	0.6	< 0.05	0.1	1.2	1.4
28	*Sebacinales Group B sp*	SH179096.07FU	nd	0.01	0.01	nd	1.8	1.4	nd	< 0.01	< 0.01	nd	< 0.01	< 0.01	< 0.01
29	*Oidiodendron sp*	SH216998.07FU	nd	< 0.01	0.6	nd	0.01	1.1	nd	0.01	0.5	< 0.05	0.1	1.1	1.3
30	*Helvella*	SH223062.06FU	< 0.05	< 0.01	0.01	nd	< 0.01	0.01	< 0.05	< 0.01	0.01	1.2	0.8	3.2	0.02

The species identification of the OTUs which utilized substantial amount (>1% of all detected sequences) of ^{13}C from all the plants in publication **I** are written in bold. The intensity of the color coding indicates the abundance of OTU in each sample type. nd, not detected; na, not available. Table updated and modified from the Table 3 in publication **I**.

4.1.2 Host preference of the root-associated fungi

The host specificity or preference of ERM fungi is well debated, and studies investigating this have obtained differing results. Some studies indicate that ERM fungi do possess host preference (Bougoure *et al.*, 2007; Ishida & Nordin, 2010; Toju *et al.*, 2016a) while others suggest that they do not (Kjøller *et al.*, 2010; Walker *et al.*, 2011). In publication **I**, the studied ericoid plants, *C. vulgaris*, *V. myrtillus* and *V. vitis-idaea*, harbored different fungal communities in their roots, suggesting that they provide different niches for the fungi.

The *R. ericae* aggregate, formed by species from genera *Rhizoscyphus*, *Meliniomyces* and *Cadophora*, is known to contain both ECM and ERM species (Vrålstad *et al.*, 2000, 2002; Hambleton & Sigler, 2005; Grelet *et al.*, 2009, 2010) and is detected from both ericoid and coniferous plant roots (Monreal *et al.*, 1999; Villarreal-Ruiz *et al.*, 2004; Heinonsalo *et al.*, 2007; Bent *et al.*, 2011; Bruzone *et al.*, 2017). Similarly, in publication **I**, members of the *R. ericae* aggregate were detected from the roots of both the ericoid plants as well as *P. sylvestris*. *R. ericae* was expectedly more abundant in ericoid roots than those of *P. sylvestris*, and correspondingly the mainly ECM-forming *M. vraolstadiae* was more dominant in the *P. sylvestris* roots (Table 2, publication **I**). However, not all of the members of the *R. ericae* aggregate were consistently present and utilizing photosynthetic-^{13}C in the corresponding ECM or ERM plant roots. The ERM fungus *M. variabilis* was interestingly more abundant in the *P. sylvestris* roots than in ericoid plant roots and it was over 4-times more abundant in the ^{13}C-labeled root community of *P. sylvestris* than ericoid plants (Table 2, publication **I**). In addition, both the ERM and ECM forming fungus, *M. bicolor*, was detected in significant amounts only from the roots and soils of *P. sylvestris*, and even there, it did not utilize photosynthetic-^{13}C from *P. sylvestris* (Table 2, publication **I**). These results indicate that in the mixed fungal community the ECM and ERM species of the *R. ericae* aggregate differ in their photosynthates utilizing abilities and affinities for colonizing the corresponding plant roots.

Based on publication **I**, the capability of the *R. ericae* aggregate species to form mycorrhizal associations with their host plants depends on the environmental conditions and surrounding fungal community. This is further supported by results from other studies. The ERM *M. variabilis* is commonly detected to live as an endophyte in roots of coniferous trees (Piercey *et al.*, 2002; Vohnik *et al.*, 2013; Heinonsalo *et al.*, 2017), and some *M. variabilis* strains have failed to form ERM structures when inoculated to ericoid roots (Piercey *et al.*, 2002; Vrålstad *et al.*, 2002; Martino *et al.*, 2018). Despite its dual ECM and ERM role, *M. bicolor* was found to be unable to form ECM symbiosis with coniferous trees roots when the conifers were co-existing with ericoid plants although the same fungal strain did form ECM when the coniferous plants were grown alone (Kohout *et al.*, 2011). Martino *et al.* (2018) suggested that the transition of the ERM fungi from a saprotrophic or endophytic lifestyle to a mycorrhizal lifestyle is continuing. Based on this hypothesis, *M. variabilis* and *M. bicolor* might have lost the competition for a

mycorrhizal niche in their host plant roots to other fungi with better competitive fitness (publication **I**).

Although the ericoid plants, *C. vulgaris*, *V. myrtillus* and *V. vitis-idaea*, shared many species with each other, their root-associated fungal community structures differed from each other, indicating that they provide different ecological niches for the ERM fungi (publication **I**). The root-associated community of *V. vitis-idaea* differed especially from the other ericoid plants. The basidiomycete fungi from order Sebacinales were over 12 times more abundant in *V. vitis-idaea* roots than in the other plant roots (Table 2 in publication **I**). The order Sebacinales is an ecologically diverse group of fungi with saprotrophic, endophytic, ECM and ERM lifestyles, and it harbors two evolutionary distinct families: Sebacinaceae and clade B Sebacinales (syn. Serendipitaceae) (Weiß *et al.*, 2016). From these, Sebacinaceae are mainly ECM and endophytic fungi whereas clade B Sebacinales include fungi capable of living in the plant roots as endophytes, saprotrophs or as putative ERM fungi forming associations with orchids and ericoids (Selosse *et al.*, 2007; Weiß *et al.*, 2011, 2016; Vohník *et al.*, 2016). Three OTUs of the clade B Sebacinales as well as one OTU from genus *Sebacina* (family: Sebacinaceae), were detected from the *V. vitis-idaea* roots and utilized photosynthetic-^{13}C from it (Table 2 in publication **I**). Interestingly, the *Sebacina* without a confirmed ERM status utilized more ^{13}C from *V. vitis-idaea* than the putative ERM fungi from Clade B Sebacinales (Table 2). However, the ecology of *Sebacina* and whether it was utilizing the photosynthetic-^{13}C as an endophyte or through traditional ERM structure remains to be clarified.

In addition to the mycorrhizal lifestyle, some fungi live in the roots as endophytes, and some mycorrhizal fungi can adapt an endophytic lifestyle when living in non-host plant roots (Sieber, 2002; Brundrett, 2002). In the roots, endophytes can have positive, neutral or negative effects for the plant growth (Haselwandter & Read, 1982; Jumpponen *et al.*, 1998; Menkis *et al.*, 2004; Addy *et al.*, 2005; Lukešová *et al.*, 2015). When co-occurring with mycorrhizal fungi, endophytes can assist plant nutrient uptake by releasing nutrients from the soil (Reininger & Sieber, 2012, 2013), without forming bilateral nutrient exchange structures with the roots (Brundrett, 2004). In publication **I,** the ^{13}C-utilizing *M. variabilis* in *P. sylvestris* roots, *Sebacina* in *V. vitis-idaea* roots, and PAC members in all the studied plants roots used the ^{13}C probably as endophytes and not through a traditional mycorrhizal interface. However, as the labeling time in publication **I** was relatively short, the ^{13}C transfer between the plant and fungus indicates that they potentially have a dynamic interaction and important role in the soil ecosystem. The specific functions and interactions with the other root-associated fungi however need to be enlightened in future studies.

4.1.3 Changes induced in the soil chemical profile by the coniferous tree and ericoid plants

In general, the ericoid plants and *P. sylvestris* had a statistically significant effect on the soil chemical profile and activities of the soil inhabiting microbial community (Figure 4 in publication **II**). Within all the plant species, the plant roots and rhizome associated microbes were capable of obtaining ^{15}N from the dead fungal mycelia and deliver it to the host plant at a similar rate (Table 2 in publication **II**). The unplanted soils had higher inorganic ammonium content than the planted soils (Figure 1 in publication **II**). The lower ammonium content in the planted soils compared to the unplanted soils in publication **II** indicates that the plants and their associated fungi have taken up N from the soil. This is further supported by the lower C/N ratio in the unplanted soil compared to the planted soils (Figure 1 in publication **II**). However, at the end of the experiment, the C/N ratio in the ericoid soils was higher than in *P. sylvestris* soils. One reason for this could be evolutionary, since the ERM and ECM fungi have preserved different organic matter degradation related genepools when evolving from saprotrophic fungi (Kohler *et al.*, 2015; Martino *et al.*, 2018). The different C/N ratios in the soils of ERM-plants and the ECM-plant (Figure 1 in publication **II**), might reflect the different approaches which the ERM and ECM fungi use to scavenge the nutrients from the humus.

Overall, the pH of the unplanted soil was higher than in the planted soils (Figure 1 in publication **II**). Based also on other studies the pH of the humus is strongly influenced by the presence of the plant roots and their exudates (Kieloaho *et al.*, 2016). In publication **II**, the soil pH declined the most by the influence of ericoid plants. Since ericoid plants and their root associated ERM fungi, are common inhabitants of harsh environments with low soil pH (Cairney & Meharg, 2003), they might have adapted to a more acidic environment. Additionally, ericoid plants and their microbial associates might have produced organic acids and other exudates to lower the pH of the surrounding soils (publication **II**).

The plant secondary metabolic compounds, especially tannins, are known to be able to precipitate proteins resulting in decreased enzymatic activity (Adamczyk *et al.*, 2015, 2017). In publication **II**, the planted soils had in general lower enzymatic activities than the unplanted soils (Figure 2 in publication **II**), which could be explained by the presence of the plants roots and their phenolic compounds. However, only *C. vulgaris* soils contained high concentrations of phenols and tannins (Figure 1 in publication **II**). Therefore, it is likely that also other factors, such as soil pH, affected the measured enzyme activities of the soils. As the plants and their associated microbes lowered the pH of the surrounding soils, they might have also produced organic matter degrading enzymes with lower optimal pH than the ones produced by the microbial community in the unplanted soils. The enzyme activities were measured from the soils at fixed pH, which was close to the natural pH of the unplanted soils, but higher than the pH of the planted soils (publication **II**). The plant photosynthetic flow can also enhance the activity of the root-associated microbes (Kuzyakov & Blagodatskaya, 2015). In the planted soils,

the most active microbes might have been associated close to the plant roots rather than further into the soils, and as the unplanted soils lacked the photosynthetic flow, the active microbes might have been scattered throughout the soil (publication **II**).

4.1.4 Effect of the fungal community on soil chemistry

Through photosynthetic C flow and root exudates, plants affect the below-ground processes, such as the soil associated microbial community structure, microbial organic matter degrading enzyme-secretion and activity, as well as soil nutrient cycling (Kuzyakov & Blagodatskaya, 2015; Baldrian, 2017b; Huo *et al.*, 2017). However, the specific effects of the common boreal forest plants on the soil microbial community, it´s activity and the SOM chemical composition need to be further elucidated. In publications **I** and **II,** the studied plants affected the soil microbial community structure, its activity and soil chemistry differently. In addition, the conifer *P. sylvestris* had different effects on the soil fungal community structure (publication **I**), microbial enzymatic activity and soil chemistry (publication **II**) than the three ericoid plants.

All the studied plants and their microbiomes caused different changes to the chemical composition and measured organic matter-degrading enzyme activities of the surrounding soils (publication **II**). Interestingly, compared to the *C. vulgaris* and *V. myrtillus* soils, *V. vitis-idaea* and *P. sylvestris* soils had more similar pH, ammonium content, and amount of enchytraeid worms, as well as β-glucosidase and cellobiohydrolase I activities (Figures 1 and 2 in publication **II**). Furthermore, *V. vitis-idaea* and *P. sylvestris* soils clustered in the CCA closer together than the other studied soils (Figure 4 in publication **II**). Similarly, the fungal community structures of the *V. vitis-idaea* and *P. sylvestris* soils differed more from the soils of *C. vulgaris* and *V. myrtillus* ($p \leq 0.01$) than from each other, although also the *V. vitis-idaea* and *P. sylvestris* soils harbored different fungal communities ($p \leq 0.05$) (Supplementary table S3 in publication **I**). The differences in the soil fungal community structures (publication **I**) could have affected the soil chemical profile and enzymatic activities (publication **II**). There, especially the differences in the abundances of the ERM *R. ericae* (OTU 1), as well as saprotrophic *O. pilicola* (OTU 6) and Hyaloscyphaceae (OTU 21) had effect on the soils' chemical and enzymatic differences ($p \leq 0.05$) (Figure 5). In addition, the saprotrophic Dermateaceae (OTU 7) affected the soil chemical structure and enzymatic activities ($p \leq 0.05$). However, the abundance of Dermateaceae correlated highly with the abundance of *O. pilicola* and was due to this cross-correlation excluded from the CCA presented in Figure 5.

R. ericae was the most abundant in the *C. vulgaris* and *V. myrtillus* soils, less abundant in the *V. vitis-idaea* soils and rarer in the *P. sylvestris* and unplanted soils (Table 2). As *R. ericae* is known to possess and express an extensive number of organic matter degrading enzymes encoding genes (Kohler *et al.*, 2015; Martino *et al.*, 2018), this could partly explain the lower pH and lower ammonium as well as total N contents of the *C. vulgaris* and *V. myrtillus* soils compared to the other soils studied (publication **II**). However, the statistically significant effect of *R. ericae* on the soil properties in the CCA-

model (Figure 4) could be also partly due to the plant-species effect. The abundances of the saprotrophic *O. pilicola*, Dermateaceae and Hyaloscyphaceae were the highest in the unplanted control soils, followed by the soils of *V. vitis-idaea* and *P. sylvestris* being rarer in the *C. vulgaris* and *V. myrtillus* soils (Table 2). The different abundances of the three above-mentioned saprotrophs might have contributed to the detected similarities and differences between the soils of *V. vitis-idaea* and *P. sylvestris* when compared to *C. vulgaris* and *V. myrtillus* soils, as well as of the unplanted soils when compared to all the planted soils. In addition, basidiomycete fungi were more dominant in the soils of *V. vitis-idaea* and *P. sylvestris* than in any of the other studied soils (Table 2 in publication **I**). Although the abundance of the ECM *P. sphaerosporum* (OTU 9) was not a statistically significant factor explaining the observed differences in the soil properties (Figure 5), the ERM and ECM are known to possess different organic matter degradation-related gene pools (Kohler *et al.*, 2015; Martino *et al.*, 2018). Therefore, *P. sphaerosporum* might have contributed to the different soil chemical and enzymatic profiles between *P. sylvestris* and the ericoids (Figures 1, 2 and 4 in publication **II**).

The plants and their soil chemistry induced changes could correspondingly have shaped the soil microbial community. For example, soil microbial community structures are found to be highly dependent on the surrounding pH (Erland & Taylor, 2002; Rousk *et al.*, 2009). The higher total phenolics and condensed tannin contents of the ericoid roots and *C. vulgaris* soils, when compared to the unplanted controls and *P. sylvestris* (publication **II**), might also inhibit certain microbial secreted enzymes (Baldrian, 2006; Adamczyk *et al.*, 2015, 2017) and favor some microbes over others. The chemical and enzymatic profiles of *V. myrtillus* differed from those of other ericoid plants (Figure 4 in publication **II**), as did the bacterial community structure of *V. myrtillus* soils when compared to the soils of other ericoid plants (Timonen *et al.*, 2017). Since both the plant-root associated fungal and bacterial communities differed between the plant species (Timonen *et al.*, 2017; publication **I**), they might also have contributed to shaping the soil chemical profiles studied in publication **II** through their exudates and nutrient acquisition strategies. Thus, as postulated already by Baldrian (2017b), the plant-microbe interactions and the differences induced by them in the boreal forest soils are dynamic, and more emphasis should be made to study both the fungal and bacterial community structures and their activity simultaneously.

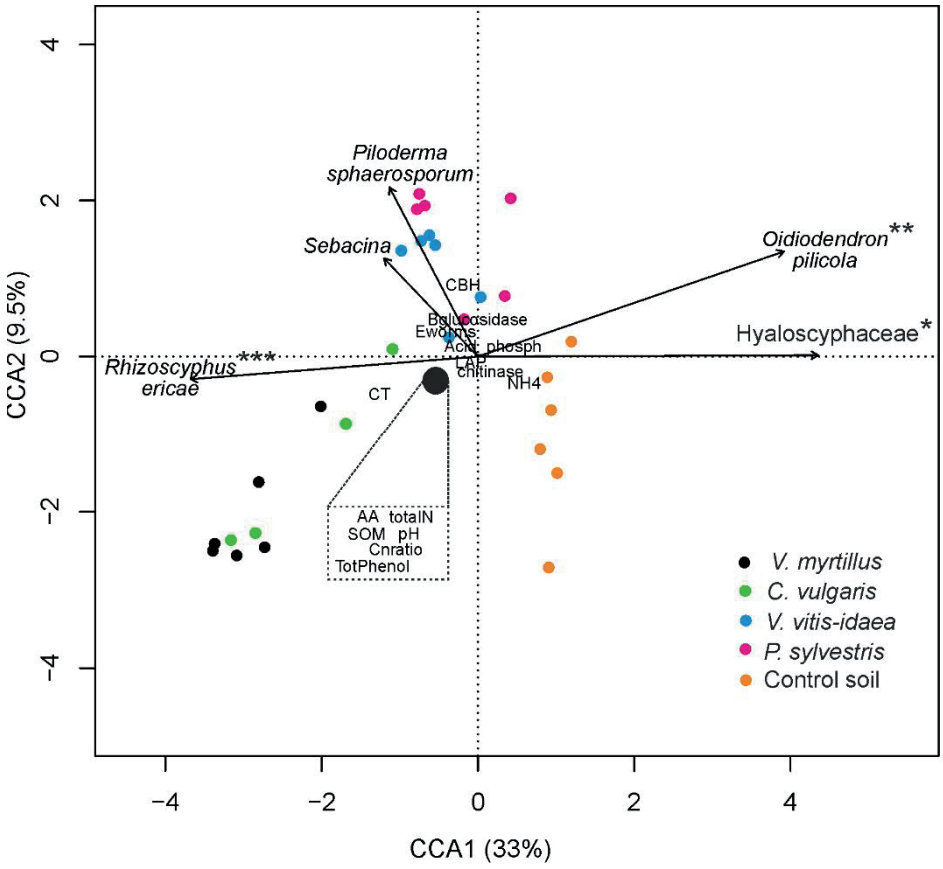

Figure 5. Canonical correspondence analysis where the soil chemical profiles and enzymatic activities measured in publication **II** are set as response variables and the explanatory variables are selected with ordistep-function from 30 most abundant soil fungi determined in publication **I**. The CCA1-axis explained 33% of the variance with statistical significance ($p \leq 0.001$), and the CCA2-axis explained 9.5% of the variance. The fungal species which explained the differentiation of the data with statistical significance are marked with asterisks; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. The Dermateaceae (OTU 7) was excluded from the CCA since it´s abundance cross-correlated with *O. pilicola* (OTU 6) presented in the CCA.

4.2 Effect of restricted carbon flow

The response of the soil fungal community to the multiyear restriction of photosynthetic C flow from plant to soil is not yet fully known. In addition, the mechanisms behind the spatial separation of saprotrophic dominance in the litter layers and ECM dominance in the more recalcitrant humus layers are still debated. Some studies indicate that this spatial separation is due to antagonism between these two fungal guilds (Gadgil & Gadgil, 1971, 1975) whereas it is also suggested that one reason behind this separation is differing niche and substrate preferences (Fernandez & Kennedy, 2016). Manuscript **III** aimed to enlighten how the restriction of direct C flow from the plants shapes the microbial communities and their potential functionality in humus. In addition, following the "Gadgil effect" theory, the interest was to observe if the dominance shifts from ECM fungi to saprotrophs in humus. For this, a three-year field experiment, where the photosynthetic C flow was manipulated with three different mesh sizes (1000 µm, 50 µm and 1 µm), was established. The manipulation of the direct C flow caused moderate changes to the fungal community structure over the three years. However, contrary to the Gadgil theory, the saprotrophs did not become more dominant in the 1 µm mesh bags without the direct photosynthetic flow (manuscript **III**). The manipulation of the plant-C flow caused minor changes also in the bacterial community structure, but abiotic factors, such as soil pH and moisture content, were more significant drivers in determining the bacterial community structures than the mesh treatments (manuscript **III**). The results indicate that the soil-associated microbial communities are rather flexible and adaptive towards relatively short-term environmental changes.

4.2.1 Response of the fungal community to restriction of photosynthetic carbon flow

Interestingly, the abundances of the ECM *Russula* and *Cortinarius*, which can produce organic matter degradation related enzymes, increased in the 1 µm mesh treatment and decreased in the 1000 µm mesh treatment during the three growing seasons (Table 2 in manuscript **III**). Overall, the 1 µm mesh bags were dominated by the symbiotic ECM and ERM fungal guilds (Figure 3 in manuscript **III**). Although the ECM and ERM fungi have lost some of their ancestral saprotrophic apparatus (Kohler *et al.*, 2015; Martin *et al.*, 2016; Martino *et al.*, 2018), some of them are able to produce SOM degradation related enzymes and are efficient scavengers of N from complex organic matter (Bödeker *et al.*, 2009, 2014, Heinonsalo *et al.*, 2012, 2015; Talbot *et al.*, 2015). The primary reason for ECM fungi to degrade the SOM is considered to be for scavenging for N (Lindahl & Tunlid, 2015), and the released C is mainly utilized by other microbes living close to the fungal hyphae (Talbot *et al.*, 2008). However, when the C flow from the plant is insufficient, the ECM fungi may use the released C themselves ("Plan B hypothesis" by Talbot *et al.* (2008)). The slight saprotrophic abilities of ECM fungi could explain the relatively high mass loss in the 1 µm mesh treatments (Supplementary Table 2

in manuscript **III**) although the 1 µm mesh bags were dominated by ECM instead of saprotrophs.

The increased access to plant photosynthates is proposed to accelerate the degradation of old and more difficultly degradable SOM through the "priming effect" (Kuzyakov & Domanski, 2000). Based on theory behind the "priming effect", when the plant photosynthetic rates and litter input decrease, the microbial community will lower their metabolic activity. Additionally, the microbes can adapt to rather short disturbances, such as nutrient depletion, by changing their gene expression patterns or by going into a metabolically inactive state (Stenström *et al.*, 2001; Jones & Lennon, 2010; Lennon & Jones, 2011). The restriction of the direct photosynthetic C flow in manuscript **III** may have caused the soil microbial community to lower their metabolic rates and to decrease the microbial SOM degradation. This could be reflected in the lower mass loss in 1 µm treatment compared to the other treatments (Supplementary Table 2 in manuscript **III**). In addition, especially the 1 µm mesh treatment sheltered the humus in the mesh bags from mixing caused by natural soil macro- and meso-fauna. Thus, if some of the microbes in the 1 µm mesh bags have been in a dormant state because of the depleted photosynthetic flow, they could have persisted there longer than in natural forest soil.

4.2.2 Effects of photosynthetic carbon flow manipulation on the bacterial community and potential functional gene pools

By transporting plant exudates or producing bactericides, fungi can shape the soil associated bacterial community (de Boer *et al.*, 2005). Many studies investigating the fungi-bacteria relationships in boreal forest ecosystem have focused mainly on the root-associated fungi and their bacterial partners (Marupakula *et al.*, 2017). However, mycorrhizal roots are observed to harbor different bacterial populations than the surrounding soil (Skyring & Quadling, 1969; Timonen *et al.*, 2017). In manuscript **III**, the bacterial populations were most affected by the soil pH and soil moisture content rather than the fungal population or the treatment. These findings are in line with previous observations, according to which the soil properties are the most important factors shaping the soil-associated bacterial community (Männistö *et al.*, 2007; Jeanbille *et al.*, 2016). However, in manuscript **III**, some of the differences in the soil chemistry may have been caused by the restriction of the direct photosynthetic C flow into the mesh bags. Based on previous studies, the pH of humus is strongly influenced by the presence of plant roots and exudates, and the pH of unplanted humus soils can rise over pH 4.5 while the pH is typically around 4.0 (Kieloaho *et al.*, 2016; publication **II**). This phenomenon was visible also in the mesh treatments (Supplementary Table 2 in manuscript **III**), where the pH of humus soils inside the 1000 µm and 50µm mesh bags declined from 4.5 to 4.0 during the experiment, and the pH was constantly higher in 1 µm mesh bags than in other mesh sizes. Thus, the observed changes in soil pH and the influence of the pH differences on the microbial communities in manuscript **III** could have been partly mediated by the exclusion of plant roots.

The restriction of photosynthetic C flow did not cause significant differences to the potential functional gene pools between different mesh sizes (manuscript **III**). Typically fungi and bacteria contain multiple copies of genes encoding organic matter degradation related enzymes (Floudas *et al.*, 2012; López-Mondéjar *et al.*, 2016; Martino *et al.*, 2018), and it is probable that only drastic changes in microbial community structures will affect the community's potential functionality. In addition, the study by Baldrian *et al.* (2012) revealed that the structure of the active microbial community in boreal forest soil differs highly from that of the total microbial community. In manuscript **III**, the genes encoding for SOM degradation related enzymes were probably expressed differently and by different individuals in different mesh treatments. However, to confirm this, further study investigating how the manipulation of the photosynthetic C flow affects the microbial community's RNA- and DNA-profiles and comparing the community's true (RNA-based) and potential (DNA-based) functionality is needed.

4.2.3 Substrate preferences of saprotrophic and ectomycorrhizal fungi?

In manuscript **III**, contrary to the "Gadgil effect" theory and previous findings (Gadgil & Gadgil, 1971, 1975; Lindahl *et al.*, 2010; Bödeker *et al.*, 2016), the abundance of fungi from saprotrophic guild did not increase in the 1 µm mesh bag treatments, in which the direct C flow from the plant was restricted (Figure 3 in manuscript **III**). Actually, at the end of the three-year experiment, the abundances of universal and wood saprotrophs were lower in the 1 µm mesh treatment than in the 1000 µm mesh treatment, which simulated the normal soil conditions (p-adj $\leq$ 0.05 with DESeq2, manuscript **III**). Most of the experiments describing the competition between the saprotrophs and ECM are conducted in litter layer (Gadgil & Gadgil, 1971, 1975; Koide & Wu, 2003; Bödeker *et al.*, 2016), microcosms (Lindahl *et al.*, 1999, 2001), or by restricting the photosynthetic flow by cutting the plant roots but leaving the roots in the soil (Lindahl *et al.*, 2010). Consequently, in all these experiments, there has been plant biomass left to serve as a substrate for the saprotrophs. As proposed by Averill & Hawkes (2016) and Fernandez & Kennedy (2016), besides the exclusion of the photosynthetic C flow, the magnitude and presence of the "Gadgil effect" is mediated also by the increase of labile-C input to soils due to the disturbance of the treatment. In manuscript **III**, the mesh bags contained limited amounts of plant biomass which the saprotrophs could use for substrate, since the humus inside the mesh bags was sieved to remove the fine roots and all particles larger than 4 mm. In addition, the fine roots could grow inside the 1000 µm control treatment (manuscript **III**), and after three growing seasons the fine roots were detected in all the 1000 µm mesh treatments covering up to 2% of the total fresh weight of the mesh bag (data not shown), where saprotrophs may have used them as a source of C.

Humus is a difficult substrate for the microorganisms since it contains complex polymers and a limited amount of easily available C and N (Schulten & Schnitzer, 1998; Korhonen *et al.*, 2013; Clemmensen *et al.*, 2013). The degradation of humus is suggested

to be unbeneficial for fast growing saprotrophs as the synthesis and secretion of the organic matter degrading enzymes would require more energy than they could gain from this complex substrate (Baldrian, 2009). Although the ECM fungi have less efficient saprotrophic machineries than the saprotrophs (Kohler *et al.*, 2015; Martino *et al.*, 2018), the slow growing ECM fungi are found to dominate the decomposition of the complex SOM (Fontaine *et al.*, 2003) suggesting that they have an affinity for this substrate. These differences in substrate preferences might have given the ECM fungi a competitive advantage against the saprotrophs in the 1 µm mesh bags (manuscript **III**). Similarly, the studies from Lindahl *et al.* (2001) and Bödeker *et al.* (2016) indicate that the result of the competition between mycorrhizal and saprotrophic fungi depends on the substrate and its quality.

Fernandez & Kennedy (2016) proposed that one reason for the spatial separation of saprotrophs and mycorrhizal fungi is their different niche preferences. This hypothesis is further supported by the recent field experiments (Bödeker *et al.*, 2016; manuscript **III)**. Bödeker *et al.* (2016) examined how 50 µm mesh bags filled with sterilized fresh litter, partly decomposed litter or humus were colonized by fungi from different functional guilds when the mesh bags were buried in different soil horizons. When the sterilized fresh and partly decomposed litter bags were placed inside the O_L and O_F layers, they were colonized more eagerly by saprotrophs, but when placed inside the O_H layer, saprotrophs dominated the fresh litter bags but not the partly decomposed litter bags (Bödeker *et al.*, 2016). In addition, the sterilized humus was more eagerly colonized by mycorrhizal fungi than saprotrophs no matter if the mesh bags were placed inside the litter or humus layers (Bödeker *et al.*, 2016). Similarly, the saprotrophs in humus were not given a competitive advantage over ECM with the restriction of photosynthetic C flow (manuscript **III)**. Therefore, the appearance of the "Gadgil effect" might be substrate dependent and reflect the different niche preferences of these two fungal guilds.

4.3 Methodological aspects

When studying the different wide aspects of the boreal forest biome, the experimental design and methods usually have different advantages and limitations, which are important to acknowledge during the design of the experiment and interpretation of the results. The main aspects, limitations and advantages of the experimental designs and methods used in this PhD thesis work are discussed below.

4.3.1 Laboratory-scale and field-scale experiments

This PhD thesis consisted of both laboratory-scale and field-scale experiments. In the laboratory scale study described in publications **I** and **II**, the studied plants were grown in microcosms under controlled conditions. In addition, since the homogenized humus, used for construction of the microcosms was unsterilized containing the natural soil microbiota, similar initial microbial inoculum was used in each microcosm. By growing the plants in this artificial system, it could be assured that all the plants were exposed to the same soil microbes, obtained the same amount of light, were growing in the same moisture conditions, and were of the same age and in the same growth-phase at the end of the experiment. This allowed for the detection of clear differences in the roots and soils of the different plant species, from which some would have been masked by the spatial heterogeneity present in the natural ecosystem (Dutilleul, 1993; Pickles *et al.*, 2010).

This microcosm experiment was designed to address the individual effects caused by the studied plant species (publications **I** and **II**). However, Kohout *et al.* (2011) reported that the fungal community colonizing the root tips of *Pinus* seedlings was different if the plants were grown individually or intermingled with the *Vaccinium* species. Since in publications **I** and **II** the plants were grown alone and not intermingled with other plant species, the fungal community in their roots might differ from that in natural forests. Thus, the results from publications **I** and **II** must be interpreted from the perspective that the plants were grown separately, the study was designed to investigate the individual changes driven by these studied plant species, and these findings cannot be directly extrapolated to the ecosystem level.

Laboratory-scale experiments are in some cases unsuitable to investigate the study question on an ecologically relevant scale, since some changes may occur too slowly or rapidly, or too intensively or incompletely compared to how they would occur in the ecosystem (Carpenter, 1996). In these cases establishment of a field-scale study is required, however there are some considerations that need to be acknowledged. For example, the spatial heterogeneity, i.e. variability of ecosystem properties across the studied area is an important factor to consider when planning the experimental design and interpreting the results (Hurlbert, 1984; Dutilleul, 1993). In boreal forest soils, the fungal community is observed to differ highly even in relatively small areas (e.g. Pickles *et al.*, 2010; Santalahti *et al.*, 2016), and to observe and distinguish real treatment effects from the differences caused by spatial variation, wide dispersion of multiple replicates is needed (Hurlbert, 1984; Dutilleul, 1993; Dutilleul & Legendre, 1993). In manuscript **III**,

the mesh bags were evenly distributed to three different sites at least 50 m apart from each other in Hyytiälä forestry field station used previously by Santalahti *et al.* (2016). Although the forest at these sites is visually of uniform quality, both in the study by Santalahti *et al.* (2016) and in manuscript **III**, the fungal community structures in these three experimental sites differed from each other with statistical significance. However, despite this spatial variation, general trends caused by treatment could be observed (manuscript **III**). As these trends were visible in all areas, the findings are ecologically more relevant than if the experiment would have been conducted using a single experimental site.

As the laboratory-scale and field-scale experiments both have their own advantages and disadvantages, the selection of the appropriate method for addressing the study-question is critical. In some cases, the aim can be reached only in controlled conditions without natural heterogeneity, and in some cases, ecologically relevant results can be obtained only by studying the question of interest in field conditions. When designing the experimental setup, these limitations and benefits must be considered.

4.3.2 DNA-based stable isotope probing technique

The DNA-based molecular techniques can detect both the mycorrhizal as well as non-mycorrhizal opportunistically root-colonizing fungi (Leopold, 2016; Vohník *et al.*, 2016; Grunewaldt-Stöcker & von Alten, 2016). For understanding the nutritional status of the plant root-associated fungi, the occurrence of nutrient and C transfer between the fungus and the plant are investigated (Simard *et al.*, 1997; Deslippe *et al.*, 2016). For detecting the C and nutrient transfer between the plant and fungus, DNA- and RNA-based stable isotope probing (DNA-SIP or RNA-SIP) techniques can be utilized. The DNA-SIP allows for detection of those microbes which have taken up the labeled substrate and utilized it to construct or repair their genomic DNA (Dumont & Murrell, 2005). In publication **I**, the DNA-SIP was used to detect those plant root-associated fungi which utilized the recently photosynthesized-^{13}C. For this, the plants were exposed to $^{13}CO_2$ two hours per day in five adjacent days followed by a 3–4 day lag period. During this time the plants could transport the ^{13}C-sugars to their roots and the root-associated microbes could incorporate the ^{13}C into their genomic-DNA (Högberg *et al.*, 2008).

The major risk with the ^{13}C-DNA-SIP-method used in publication **I** is that the ^{13}C-label could leak to saprotrophs or other rhizosphere-dwelling fungi after the death of plant roots or primary photosynthate utilizing microbes. The plants typically allocate the recently photosynthesized C along the plant organs based on the sink strengths (Högberg *et al.*, 2008; Lemoine *et al.*, 2013). *P. sylvestris* transports the recently photosynthesized C effectively to the root tips (Heinonsalo *et al.*, 2004) and the metabolically active roots are stronger sinks of recent photosynthates than the less active roots (Högberg *et al.*, 2008). As the labeling time in publication **I** was relatively short, it is unlikely that the dying plant roots and those degrading saprotrophs would obtain a substantial amount of the labeled ^{13}C. This is further supported by the fact that the ^{13}C-labeled ericoid roots were dominated by rather slow growing ERM fungi (Table 2, publication **I**), suggesting

that they had primary access to the recently photosynthesized ^{13}C-sugars over the rhizosphere-dwelling, and often fast growing, opportunistic or saprotrophic fungi.

The ^{13}C-DNA-SIP-method was used in publication **I** to detect carbohydrate transfer from plant to its root-associated fungi. Admittedly, the detection of only one-way nutrient transfer is inadequate evidence to confirm the mycorrhizal status of the fungus. However, as the C transfer from plant to fungus is considered to be one of the key elements of the mycorrhizal symbiosis (Smith & Read, 2008), the detection of the plant root-associated fungus utilizing photosynthetic-^{13}C in publication **I** can be regarded as an indication of dynamic interaction between the plant and fungus. The confirmation of the natures of these associations and interactions need to be verified with axenic cultures in re-synthesis tests and microscopy, and with methods tracking the bilateral nutrient exchange between the host-plant and the fungus.

4.3.3 DNA-based community analysis

The DNA-based community analysis is a powerful and widely used tool in ecology to study the microbial community structures and their sensitivity to different environmental changes, but this approach has some limitations. As the soil inhabiting microbes can be living and active, in dormant state, deceased, or as spores, the DNA-based assays are unable to discriminate if the detected microbes are active or not (Jones & Lennon, 2010; Lennon & Jones, 2011; Baldrian *et al.*, 2012; Blazewicz *et al.*, 2013; Dlott *et al.*, 2015; Carini *et al.*, 2016). Therefore, the microbial community revealed with the DNA-based methods, may be considered as the potentially active microbial community. For investigating the currently active microbial community, RNA-based community assays are recommended (Baldrian *et al.*, 2012; Dlott *et al.*, 2015). However, the methods for investigation of active microbes based on rRNA (ribosomal RNA) also have their own disadvantages, since the dormant microbes can contain numerous rRNA copies (Blazewicz *et al.*, 2013). In addition, the DNA-based community assays are also effective for detecting clear changes in the community structures, such as in publication **I**. In general, the persistence of RNA-molecules is rather short and the RNA-based assay could describe only the situation shortly before and at the time of the sampling, since it is dependent on the current environmental conditions.

With the next generation sequencing, the primers used for targeting the fungi might prefer some species and overlook others (Jumpponen, 2007; Lindahl *et al.*, 2013), possibly causing some bias to the observed community. In addition, species with more rDNA gene copies in their genomes could be deemed more abundant than the ones with less rDNA copies. For this, rDNA copy number correction could be applied (Dlott *et al.*, 2015). However, for applying this correction to the fungal community, more whole genome sequencing data would be needed. Despite these uncertainties, DNA-based community analysis is still a valuable tool for studying the fungal communities and their structures, since it allows the detection of non-cultivable fungi (Allen *et al.*, 2003; Jumpponen, 2007).

5 Conclusions

In this PhD thesis, both laboratory- and field-scale studies were combined to investigate the importance of plant-derived photosynthates in shaping fungal community structures and driving SOM decomposition in the boreal forest ecosystem (publications **I** and **II**, manuscript **III**). Furthermore, the root-colonizing fungi, which utilized recently photosynthesized-C from the studied three ericoid plants and a coniferous tree, were identified using ^{13}C-DNA-SIP (publication **I**).

The ericoid plants *C. vulgaris*, *V. myrtillus* and *V. vitis-idaea*, as well as the coniferous tree *P. sylvestris*, shaped their root-associated fungal community structures (publication **I**). In general, the plants had a more intensive effect on the fungal community in the plant roots than in the surrounding soils. The three ericoid plants shaped their soil and root-associated fungal communities differently than the conifer *P. sylvestris*, and the ericoid plants favored different fungal communities in their roots. The results suggest that these four studied plants provided in their roots and surrounding soil various niches which different fungi preferred. Altogether, those fungi, which were the most dominant in the roots of *C. vulgaris*, *V. myrtillus*, *V. vitis-idaea* and *P. sylvestris*, also utilized most intensively the recently photosynthesized ^{13}C. The ECM *P. sylvestris* and the three ERM-plants shared many fungal species in their roots, and some of these fungi utilized recent photosynthates from all of the hosts.

The different ericoid plants and their associated microbes affected the SOM decomposition and nutrient cycling in humus similarly (publication **II**). The ericoid plant roots contained more phenolic compounds and the soil pH of ericoid planted soils decreased more compared to *P. sylvestris* roots and soils. These can cause significant differences in the soil chemical composition as well as microbiome functionality. The ECM *P. sylvestris* and the studied ERM-plants *C. vulgaris*, *V. myrtillus* and *V. vitis-idaea* shaped the soil organic and inorganic N-pools similarly, and their associated fungi delivered N to their hosts at similar rates. However, the C/N ratio in the ericoid planted soils increased more than in the *P. sylvestris* planted soils. As the ERM and ECM fungi have preserved different organic matter degradation related genepools when evolving from saprotrophic fungi, the different C/N ratios might be due to the different approaches they use to scavenge the nutrients from the complex SOM.

As the conifer *P. sylvestris* and its associated fungi share the same ecological habitats as the ericoid plants and their associated fungi, it is important to gain more knowledge on the belowground processes that these plants drive, and their dynamics, in the boreal forest ecosystem. For this, the ecological role of the co-occurrence, co-functioning and the nature of the various interactions of the plant root-associated ERM, ECM and endophytic fungi should be studied more in the future.

The manipulation of the direct photosynthetic C flow from the plant caused moderate changes to the fungal community structures in humus, and lower mass loss to treatments where plant C flow was restricted than in the controls (manuscript **III**). The results

suggest that members of the boreal forest soil fungal community are rather flexible towards short-term disturbances, but their activity and functionality might change due to the disturbance. Contrary to the "Gadgil effect" theory, the restriction of plant C flow did not shift the dominance from ECM to saprotrophs in the humus. Thus, the results support the hypothesis that the mechanisms behind the "Gadgil effect" are more complex and are not limited only to the competition for C. In addition, the spatial differentiation of ECM and saprotrophic fungi might be driven by affinity towards different substrates rather than competition between guilds. For predicting how the belowground microbial community and it´s functionality shifts in a response to the changing climate, more long-term experiments assessing simultaneously the differences induced in both the total community and active community are necessary in the future.

This PhD thesis provided novel information on the importance of different plant species in shaping the root- and soil-associated fungal community structures as well as the soil chemical profile. In addition, this PhD thesis provided further knowledge of the role of plant photosynthates on soil microbial community structures and the dynamics between microbes with different nutritional strategies. As the climate changes, the belowground C input from plants into soil is predicted to increase and change soil microbial processes. Based on the "priming effect" theory, the warming climate and longer growing seasons may enhance humus decomposition, and thereby decrease soil C storage. However, the increase in plant litter and microbial necromass production may also build up in SOM and increase the soil C storage. The results of this PhD thesis emphasize that the effect of plant C flow on changes to soil fungal community structure and fungal guild distribution is not yet fully understood. For predicting how the changing climate affects the soil processes in the boreal forest ecosystem more knowledge on the dynamics of plant-microbe and microbe-microbe interactions is required.

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Outi-Maaria

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