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Karoliina Huusko

DYNAMICS OF ROOT-ASSOCIATED FUNGAL COMMUNITIES IN RELATION TO DISTURBANCE IN BOREAL AND SUBARCTIC FORESTS

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DYNAMICS OF ROOT-ASSOCIATED FUNGAL COMMUNITIES IN RELATION TO DISTURBANCE IN BOREAL AND SUBARCTIC FORESTS

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Abstract

Disturbance may shift microbial communities from one state to another. However, species differ in their ecological characteristics and their abilities to withstand disturbance. No single species or individuals of a species exist alone, but they are parts of complex interaction networks including species above- and belowground. In boreal and subarctic forests, almost all plants and a high number of fungi form mycorrhizas at the plant roots. In mycorrhiza, the fungal partner harvests nutrients for the host plant and, in return, gains carbon from the plant. In general, these common associations benefit both partners, but as heterotrophs, fungi are dependent on carbon photosynthesized by plants, whereas plants can survive alone as autotrophs. In addition to mycorrhizal fungi, also other fungi, such as endophytes, saprotrophs and pathogens, live in and on plant roots.

This thesis concerns the impacts of disturbance on fungi living in plant roots and in soil near the roots. I hypothesized that i) root-associated fungal (RAF) and soil fungal communities and colonization types change after disturbance, that ii) the observed shifts relate to disturbance intensity and that iii) they co-occur with changes in soil conditions and vegetation. Changes in RAF were studied as changes in root fungal colonization, or in fungal community composition. The latter were detected with next-generation sequencing methods.

The responses of RAF to disturbance seemed to be context dependent and related to sources of fungal communities (e.g. soil, RAF networks), environmental conditions (e.g. soil pH and nutrients) and host performance. It seems that abundances of those RAF species, which are present in the roots first (priority effect), may be increased by disturbance. Research produced new information related to ecological roles of the genera *Phialocephala* and *Meliniomyces*. Altogether, the results indicate connections between both abiotic and biotic environments and RAF, and host species viability and RAF.

Keywords: 454-pyrosequencing, Betula pubescens ssp. czerepanovii, Deschampsia flexuosa, diversity, Ion Torrent sequencing, Picea abies, succession

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Tiivistelmä

Häiriöt voivat siirtää eliöyhteisön tilasta toiseen. Lajien ominaisuudet ja häiriönsietokyvyt eroavat toisistaan. Mikään laji tai yksilö ei elä yksin, vaan lajit ovat osa maan ylä- ja alapuolelle ulottuvia monimutkaisia vuorovaikutusverkostoja. Boreaalisissa ja subarktisissa metsissä lähes kaikki kasvit ja useat sienet muodostavat sienijuuren eli mykorritsan. Mykorritsassa sieniosakas hankkii isäntäkasville ravinteita ja saa vastavuoroisesti kasvilta hiiltä. Tavallisesti nämä vuorovaikutussuhteet hyödyttävät molempia sienijuuren osakkaita, mutta toisenvaraisina (heterotrofeina) sienet ovat riippuvaisia kasvien yhteyttämästä hiilestä, kun taas tuottajina (autotrofeina) kasvit voivat elää itsenäisesti. Mykorritsasienten lisäksi kasvien juurissa elää yleisesti myös muita sieniä kuten endofyyttejä, saprotrofeja ja patogeeneja.

Tämä väitöskirja käsittelee häiriön vaikutuksia sieniin, jotka elävät kasvien juurissa ja juuria ympäröivässä maassa. Hypoteesieni mukaan i) juurissa ja maassa elävien sienten yhteisöt ja kolonisaatiotyypit muuttuvat häiriön jälkeen, ii) muutokset liittyvät häiriön voimakkuuteen ja iii) muutokset tapahtuvat samanaikaisesti maan olosuhteiden ja kasvillisuuden muutoksien kanssa. Juurissa elävien sienten esiintymisen muutokset tutkittiin sienten kolonisaation tai yhteisörakenteen muutoksina. Sieniyhteisöt selvitettiin NGS-menetelmien avulla.

Juurissa elävien sienten vasteet häiriöön vaikuttavat olevan tilannesidonnaisia ja liittyvän sienilajien lähteisiin (esim. maa, juurisieniverkostot), ympäristömuuttujiin (esim. maan pH, ravinteet) ja isäntäkasvin menestymiseen. Häiriö voi vahvistaa juurissa ensimmäisenä läsnä olevien sienilajien menestymistä (prioriteettivaikutus). Uutta tietoa tuotettiin *Phialocephala* ja *Meliniomyces* –sienisukujen ekologiasta, jota tunnetaan huonosti. Kaiken kaikkiaan, tulokset osoittavat yhteydet sekä elottoman ja elollisen ympäristön ja juurten sieniyhteisön että isäntäkasvin elinkyvyn ja juurten sieniyhteisön välillä.

Asiasanat: 454-pyrosekvensointi, Betula pubescens ssp. czerepanovii, Deschampsia flexuosa, diversiteetti, Ion Torrent -sekvensointi, Picea abies, sukkessio

… sen elämän tarkoitus on jatkaa elämää ja olla

- Jukka Nousiainen,
Kohti kaukaista tähteä

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Oulu, September 2017 Karoliina Huusko

Abbreviations

AAS	atomic absorption spectrometer
AM	arbuscular mycorrhiza, arbuscular mycorrhizal
DSE	dark septate endophyte, dark septate endophytic
ECM	ectomycorrhiza, ectomycorrhizal
ERM	ericoid mycorrhiza, ericoid mycorrhizal
IDH	intermediate disturbance hypothesis
LOI	loss of ignition
NGS	next-generation sequencing
NMDS	non-metric multidimensional scaling
OTU	operational taxonomic unit
PAC	*Phialocephala-Acephala* species complex
RAF	root-associated fungi, root-associated fungal
SOM	soil organic matter

Original publications

This thesis is based on the following publications and the manuscript, which are referred throughout the text by their Roman numerals:

I Huusko, K., Ruotsalainen, A. L., & Markkola, A. M. (2017). A shift from arbuscular mycorrhizal to dark septate endophytic colonization in *Deschampsia flexuosa* roots occurs along primary successional gradient. *Mycorrhiza*, 27(2), 129–138. doi:10.1007/s00572-016-0736-x

II Huusko, K., Ruotsalainen, A. L., Wäli, P. R., Andersson, T., Koivuniemi, H., Saravesi, K., Suokas, M., Suominen, O., & Markkola, A. M. Controlled natural and simulated herbivory show contrasting effects on soil and on root fungal communities in subarctic mountain birch forest. *Manuscript*.

III Huusko, K., Tarvainen, O., Saravesi, K., Pennanen, T., Fritze, H., Kubin, E., & Markkola, A. (2015). Short-term impacts of energy wood harvesting on ectomycorrhizal fungal communities of Norway spruce saplings. *The ISME Journal*, 9(3), 581–591. doi:10.1038/ismej.2014.154

Author's contribution: K. Huusko participated in planning and establishing the experiments (I, II), field work (I, II, III) and nutrient analyses (I, II). She carried out the laboratory work required to study fungal colonization (I) and fungal communities in roots (II, III) and in soil (II). She performed bioinformatics in papers II and III, and statistics in papers I-III. She was the responsible author for all papers (I, II, III).

Contents

1 Introduction

All organisms affect and adapt to their environment. In addition to abiotic factors, the environment is modified by biological interactions between species and individuals varying from negative to positive. Clearly, heterotrophs are dependent on the autotrophs able to photosynthesize carbon, but also heterotrophs are pivotal to autotrophs, e.g. belowground microbes, especially symbiotic fungi associated with plant roots (Bever et al., 2010; van der Putten et al., 2013). Almost all boreal and subarctic plants form mycorrhizal (modern Latin *myco-* 'of fungi', Greek *-rhiza* 'root') associations with fungal symbionts (Smith & Read, 2008). Mycorrhizas are crucial in northern forest ecosystems, where soil nutrients are largely in recalcitrant forms, and not directly available to plants. However, many mycorrhizal fungal species are able to use enzymatic degradation of organic matter in their nutrient uptake (Read & Perez-Moreno, 2003). While nutrient acquisition of the host plants is increased through mycorrhizal fungi, fungal partners gain carbohydrates from their hosts in return (Smith & Read, 2008). Another relevant group of fungi in boreal and subarctic soils is saprotrophs. In general, fungi maintain many key functions in forest ecosystems, e.g. working as decomposers of organic matter in low pH forest soils, as a route for carbon to belowground and in stabilization of soil (Clemmensen et al., 2015; Fernandez & Kennedy, 2016; Moore, Robson, & Trinci, 2011).

Impacts of climate change can be expected to become stronger in the next decades (IPCC, 2014). Even though boreal forests have been exploited by us (humans) in intensive forestry for well over a century, we do not have a full understanding of how important biodiversity and species interactions are for the forest functions (Burton et al., 2010; Gauthier, Bernier, Kuuluvainen, Shvidenko, & Schepaschenko, 2015). Climate change is a global phenomenon, but its impacts can vary locally depending on the site conditions and species present (Chakraborty, Pangga, & Roper, 2012; Johnson, Angelard, Sanders, & Kiers, 2013). For example, impacts of climate change are different in tropical, temperate or subarctic forests. Especially in the northern hemisphere the growing season of fungi and plants may be extended, which may mix periodic cycles of species and their interaction (Garcia, Cabeza, Rahbek, & Araújo, 2014; Moore et al., 2011). This kind of indirect effects of climate change could be possibly seen in subarctic mountain birch forests, where a change in cyclic occurrence of insect herbivore species, e.g. due to higher winter temperatures (Jepsen, Hagen, Ims, & Yoccoz, 2008), could have cascading impacts on plant species and their microbial symbionts (Saravesi et al., 2015; Treu et al.,

2014) and nutrient cycling (Kaukonen et al., 2013) in soil. As a consequence of expanding growing seasons, carbon (C) cycles may change, as the carbon input to and outflow from soil increase (Clemmensen et al., 2015; Treseder, Marusenko, Romero-Olivares, & Maltz, 2016). Without a thorough understanding of what kinds of organisms live in forest soil and how they function, future scenarios on the effects of climate change are difficult to formulate. In the past decades, molecular tools have developed fast, which have enabled detection of belowground microbial diversity (Shokralla, Spall, Gibson, & Hajibabaei, 2012; Tedersoo et al., 2014).

1.1 Fungal guilds in roots and soil

To understand processes and patterns of fungal diversity, fungi can be classified, in addition to their taxonomy, according to their functions or habitats into different guilds. All fungi are heterotrophs, which depend on carbon photosynthesized by the autotrophs. However, fungi occupy different niches in multiple parts of ecosystems. Thus, fungal guilds can be defined based on, e.g. the main source of C that fungi use (Clemmensen et al., 2015) or the habitat fungi live in (e.g. Osono, 2006; Rämä et al., 2016). Fungal functional guilds may differ based on which extent they are present in different parts of ecosystems, reflecting their main habitats. Mycorrhizal fungi are more common in the roots and saprotrophs in the litter or soil, but still, both groups are present in both environments. This thesis focuses on the fungi that occupy plant roots, including saprotrophs and pathogens, with an emphasis on taking into account the differences in the ecological characteristics these fungal guilds display in general.

1.1.1 Root-associated fungi (RAF)

Four major types of mycorrhizae are classified based on their function and structure: arbuscular mycorrhizal, ectomycorrhizal, orchid mycorrhiza and ericoid mycorrhizal types (sensu Smith & Read, 2008; var der Heijden, Martin, Selosse, & Sanders, 2015). In addition to mycorrhizal fungi, root endophytes are present in roots. In general, the term root endophytic fungi is used to describe fungi living inside the root cell wall. As such, the term could also be used to refer to fungi forming mycorrhizas. However, in this thesis I use the term endophytic fungi to refer to fungi other than mycorrhizal root fungal endophytes (sensu Sieber & Grünig, 2013).

Arbuscular mycorrhiza is the oldest (>450 million years) and the most common mycorrhizal association between land plants and fungi (Parniske, 2008). Around 80% of land plants form arbuscular mycorrhiza with a monophyletic group of fungi belonging to the Glomeromycota clade (272 species; Schüßler, 2017; 350–1000 molecular species; Kivlin, Hawkes, & Treseder, 2011; Öpik, Davison, Moora, & Zobel, 2014). In boreal forests, arbuscular mycorrhizal (AM) fungi colonize roots of herbaceous plants and grasses. Also, some woody plants (e.g. in boreal forests *Cupressaceae*, *Rosaceae* and *Salicaceae*) may live in symbiosis with AM fungi (Smith & Read, 2008). AM fungi are obligate symbionts and they cannot reproduce without their host plants (Bonfante & Genre, 2010; Smith & Read, 2008). In addition to fungal hyphae, AM fungi form coils and tree-like structures (arbuscules) inside the root cells walls (but not inside plasmalemma), and some species form also vesicles (Smith & Read, 2008). AM fungi are well known to facilitate phosphorus (P) uptake of host plants, whereas their ecological importance in nitrogen (N) uptake, especially from organic substrates, is less clear (Hodge & Storer, 2015; Smith & Read, 2008; Table 1). In return for enhanced plant P acquisition from soil, AM fungi gain recently assimilated photosynthates from the host plant through arbuscules (Smith & Smith, 2011).

Compared to the number of AM fungal species, the number of fungi forming ectomycorrhizal (ECM) associations with woody plants (e.g. in *Pinaceae*, *Betulaceae, Fagaceae,* 2% of all plant species, van der Heijden et al., 2015) is higher totalling around 25 000 species. ECM fungi form a polyphyletic group of Ascomycetes and Basidiomycetes (Smith & Read, 2008; van der Heijden et al., 2015). Three structures are characteristics of ECM roots; a sheath or mantle which encloses the root, hyphae growing between the epidermal and cortical cells (Hartig net) but not penetrating the cell walls, and outwardly growing hyphae (external mycelium) which connects the rhizosphere parts of the fungi with the soil and with the aboveground sporocarps of the fungi (Smith & Read, 2008). ECM symbiosis has been proven to have developed several times during evolution, which can be observed for example in different types of enzymes that ECM fungi produce (Lindahl & Tunlid, 2015; Martin, Kohler, Murat, Veneault-Fourrey, & Hibbett, 2016; Tedersoo, May, & Smith, 2010). ECM ancestors are found in both main saprotrophs groups: white-rot and brown-rot fungi (Lindahl & Tunlid, 2015). Indeed, also some ECM fungi are known to produce enzymes involved in degradation of organic matter and mobilization of organic nitrogen (N), and thus they may aid the host plant to acquire nutrients especially from organic sources (Bödeker et al., 2014; Phillips, Ward, & Jones, 2014; Read & Perez-Moreno, 2003;

Table 1. Properties of root-associated fungal (RAF) groups, including their plant host groups, fungal taxonomical groups, characteristics of fungal structures and potential nutrient benefits they provide to the host plants (Sieber & Grünig, 2013; Smith & Read, 2008; van der Heijden et al., 2015). The last column shows the Roman numerals of the studies included in this thesis, that deal with the named RAF groups.

RAF group	Plant host group	Fungal taxonomic group	Characteristic structures	Potential nutrient benefits to host	Study
Arbuscular mycorrhiza	most herbs, grasses and some trees	Glomeromycota	Hyphae, coils, arbuscules, vesicles	Mineral P, N?	I
Ectomycorrhiza	Pinaceae and Angiosperms (mostly shrubs and trees), some liverworts	Basidiomycota, Ascomycota	Mantle, Hartig net, external mycelium	Organic N (mainly from proteins and small compounds)	II, III
Ericoid mycorrhiza	Ericaceae, some liverworts	Mainly Ascomycota	Loose hyphal formations, coils	Organic N (also from complex compounds)	II
Root endophytes	Trees, herbs, grasses, shrubs	Mainly Ascomycota	Hyphae, sclerotial structures	?	I

Table 1). This is crucial, as N is the most limiting nutrient in boreal and subarctic areas, as N is mainly bound in soil organic matter (SOM) and more complex forms of organic N (e.g. proteins) would otherwise be poorly accessible by trees and shrubs (Hobbie & Högberg, 2012). While nutrient availability for trees is greatly improved by ECM symbionts, ECM fungi also consume a considerable amount of photosynthetic C from trees (Högberg et al., 2008; Smith & Read, 2008). ECM fungi form a route for C fixed recently by their tree hosts to soil and to other soil biota (Clemmensen et al., 2013).

Ericoid mycorrhizal (ERM) symbiosis is not as well understood as AM and ECM symbiosis. ERM fungi form symbiotic associations with Ericaceae (*Erica, Calluna, Vaccinium, Rhododendron*). In boreal and subarctic forests, ERM fungi are common symbionts of understory dwarf shrubs (Grelet, Martino, Dickie, Tajuddin, & Artz, 2016). Most well-known ERM fungi belong to the Helotiales (Ascomycota) (Grelet et al., 2016; van der Heijden et al., 2015). ERM fungi grow as loose hyphal formations outside of the distal hair roots of their host and form coil structures inside the roots as the epidermal cells differentiate (Smith & Read, 2008). ERM fungi are known to produce enzymes that can break down recalcitrant forms of organic N (Smith & Read, 2008; Table 1), and some of ERM fungi are known to have considerable saprotrophic capabilities (Read & Perez-Moreno, 2003; Rice & Currah, 2006). Some of ERM fungi can form both ectomycorrhiza with trees and ericoid mycorrhiza with dwarf shrubs (Grelet, Johnson, Vrålstad, Alexander, & Anderson, 2010; Hambleton & Sigler, 2005; Villarreal-Ruiz, Anderson, & Alexander, 2004; Vrålstad, Myhre, & Schumacher, 2002).

Root fungal endophytes mainly belong to the Ascomycota (Sieber & Grünig, 2013; Table 1). Some of these fungi may live independently as saprotrophs in soil or colonize also plant parts other than roots. It is likely, that all plants have some fungal endophytes in their roots. One of the most-studied groups of root fungal endophytes are dark septate endophytic (DSE) fungi and the *Phialocephala-Acephala* species complex (PAC, Grünig, Queloz, Sieber, & Holdenrieder, 2008; Sieber & Grünig, 2013) within DSE. These fungi are known to colonize the roots of plant species in various environments, also in infertile soils or otherwise harsh conditions (García, Mendoza, & Pomar, 2012; Jumpponen & Trappe, 1998; Mandyam & Jumpponen, 2005; Ruotsalainen, Markkola, & Kozlov, 2007). In boreal and subarctic forests, root endophytic fungi (including DSE) are found in the roots of grasses, herbs, dwarf shrubs and trees (Sieber & Grünig, 2013). DSE fungi grow in roots as melanized septate hyphae, sometimes forming sclerotial structures or hyaline septate endophyte -type hyphae (HSE, see Barrow & Aaltonen, 2001; Yu, Nassuth, & Peterson, 2001). They do not form specific structures for carbon and nutrient exchange inside the root (Newsham, 2011). Ecological roles of DSE are not fully defined. Their impacts on the nutrient content and growth of the host have been reported to vary from positive (Newsham, 2011; Ruotsalainen & Kytöviita, 2004; Zijlstra et al., 2005) to neutral or negative (Mayerhofer, Kernaghan, & Harper, 2013).

Also fungi that form orchid mycorrhizas may be present in RAF communities. These fungal species typically live also as saprotrophs in soil or form ECM/endophytic associations with trees (van der Heijden et al., 2015).

1.1.2 Saprotrophs and pathogens

Fungal nutritional modes can be divided in three groups: saprotrophs, necrotrophs and biotrophs (Moore et al., 2011). Most of the fungi belong to saprotrophs, which fulfil a major fraction of their C needs by degradation of dead organic materials. Thus, they are the principal decomposers of wood and litter in acidic soils (van der Wal, Geydan, Kuyper, & Boer, 2013). Fungal saprotrophs belong to the Ascomycota, Basidiomycota and some to Zygomycota (Moore et al., 2011). Fungal biotrophs (e.g. mycorrhizal fungi and mutualistic fungal endophytes) exploit the host plant's cells, without killing them, and gain carbon from the host. Necrotrophs (e.g. pathogens) invade living cells, killing and utilizing them as a C source. Saprotrophs and necrotrophs have generally large habitat ranges, whereas biotrophs may be host-specific (Moore et al., 2011). In temperate and boreal forest soils, saprotrophs are the most common in the upper layers of the soil where the amount of litter and organic matter is high (Baldrian et al., 2012; Lindahl et al., 2007; O'Brien, Parrent, Jackson, Moncalvo, & Vilgalys, 2005). Fungal wood saprotrophs are generally grouped according to the decay type they form. The two most common groups are "brown rot" and "white rot" (Baldrian, 2008). Both ECM and ERM fungi have saprotroph ancestors, which can be seen in their capability to degrade some organic nutrients. Saprotrophs and pathogens can also exist in roots, and therefore the occurrences of these fungi and RAF can overlap.

1.2 Root-associated fungal (RAF) networks

Mycelium of RAF growing outwards from hosts' roots form fungal networks in soil. In addition to mycorrhizal species, roots of different plant hosts may be colonized by the same fungal endophytes, and different kinds of saprotrophs and pathogens, all of which occupy the soil around the roots. In general, some stratification between fungal guilds is seen in soil according to the main habitat of fungi. Mycorrhizal roots of the trees and other plants are more common in the deeper soil layer, where the tree roots are, whereas litter saprotrophs are found in the upper layers (Lindahl et al., 2007). Networks formed by RAF may connect root systems of different host plant species, and even carbon and nutrients may move through them from one system to another (Klein, Siegwolf, & Körner, 2016; Simard et al., 2012). In addition, to plants and fungi, other soil microbes, soil fauna, and some herbivores live in the soil having relevant roles for ecosystem functioning (Bardgett, 2005).

Interactions between organisms vary from positive to neutral and negative, also in the vicinity of the roots. Organisms occupy different niches and represent different ecological strategies. In addition to mycorrhizal types, plant species in the same community may differ in their mycorrhizal flexibility (Moora, 2014). There are plant species whose roots are not colonized by mycorrhizal fungi (non-mycorrhizal), and those whose roots are consistently (obligate mycorrhizal) or sometimes but not always colonized by mycorrhizal fungi (facultative mycorrhizal). In natural conditions, ECM and ERM are ecologically obligate partnerships for plants, whereas both obligate mycorrhizal and facultative mycorrhizal plant species associate with AM fungi (Moora, 2014). From a fungal perspective, AM fungi are obligate mutualists with a wide host range (Bonfante & Genre, 2010), whereas ECM and ERM fungi have some saprotrophic abilities (Baldrian, 2009; Grelet et al., 2016). However, in an ecological setting ECM fungi are obligate biotrophs.

Although, organisms adapt to their environment individually, their interactions and biotic parts of their surroundings are modified by other organisms. The same fungi may form associations with multiple hosts and the same host plant with multiple RAF. Both host and fungal partner can benefit in more some associations than the others (Bever et al., 2010). These kinds of association networks increase interdependence between species. Organisms and their interactions with other organisms are affected by changes in environmental conditions, species interactions and their own physiology. Even the closest associations with mycorrhizal fungi might be considered to be parasitic on plants when the net cost of the symbiosis exceeds net benefits (Hoeksema et al., 2010; Johnson, Graham, & Smith, 1997).

The roles of RAF networks in ecosystem functioning have been studied and discussed a lot recently (see the section 1.1.1.). RAF networks are known to be important for forest regeneration and seedling establishment. During forest regeneration, tree seedlings often gain ECM inoculum from belowground fungal networks associated with and kept alive by mature trees (Dickie, Koide, & Steiner, 2002; Nara, 2006; Teste, Simard, Durall, et al., 2009; van der Heijden & Horton, 2009). Seedlings that germinate on more disturbed or open sites (e.g. further away from the forest edge) are more likely to gain root fungal symbionts that originate from fungal propagules in soil and wind-dispersed spores (Teste, Simard, & Durall, 2009). Roles of endophytic fungal species (e.g. PAC) in seedling establishment are less clear (Mayerhofer et al., 2013; Sieber & Grünig, 2013; Tellenbach, Grünig, & Sieber, 2011). Little is known about how massive disturbances in soil or host carbon resources affect formation of fungal associations of seedlings and further regeneration and recovery of forests (Karst et al., 2015).

1.3 Succession-disturbance dynamics of plant and RAF communities

Succession refers to temporal changes in composition or structure of a group of organisms of different species at a site (Pickett & Cadenasso, 2005; Pickett, Meiners, & Cadenasso, 2011). Successional drivers can be divided into three categories: i) site conditions and history, ii) species availability and iii) species performance (Meiners, Cadotte, Fridley, Pickett, & Walker, 2015; Pickett & Cadenasso, 2005; Pickett et al., 2011). The spatial and temporal scales on which these drivers take place, differ from each other (Dini-Andreotte, Stegen, van Elsas, & Salles, 2015; Teste & Dickie, 2016). Clearly, species performance changes faster than site history. Climate has wider-scale impacts, whereas species interactions occur on smaller scales. Successional systems can be divided into primary and secondary, based on the lack or presence of established soil (Meiners, Pickett, & Cadenasso, 2015).

Primary succession starts in a place where no vegetation and organic soil have previously existed, and in the beginning, it is determined by abiotic factors (i). As vegetation develops and the number of autotroph and heterotroph species increases (depending on ii), biotic factors start to impact on succession more (iii). Along with succession, interactions between autotroph and heterotroph species become web-like and more complex (changes in ii and iii). Also, soil structure and nutrient resources change (i).

Secondary succession is typically started by a disturbance. Disturbance refers to an event that alters the structure of vegetation or the substrate the vegetation is growing on, often resulting in a change in resource availability (i) (White & Jentsch, 2001). To understand the impacts of disturbances, their characteristics, e.g. source, time and spatial scales, intensity and specificity, should be acknowledged (Dornelas, Soykan, & Ugland, 2011). In general, the main division is made between anthropogenic and natural disturbances, but as only few, if any, pristine landscapes exist (Boivin et al., 2016), disturbances are a mix of impacts originating from multiple sources, e.g. herbivores (insects, ungulates), human (logging, clear-cutting), or natural stochastic processes (forest fires, storms). Disturbances may range from long with chronic impacts (press), to short and discrete (pulse). They may have local or regional impacts. Disturbances may destroy existing above- and belowground communities thoroughly or partially (creating patches) and alter nutrient cycling or carbon allocation. Furthermore, species vary in their ability to withstand disturbances (Dornelas et al., 2011; White & Jentsch, 2001).

RAF communities change along succession and are altered by disturbances (Smith & Read, 2008). In general, successional changes in RAF community co-occur with changes in growing conditions of these fungi: in other words, changes in autotroph community and carbon sources (e.g. Clemmensen et al., 2015), soil structure, water and nutrient availability and pH, species availability and species interactions (cf. Dickie et al., 2013). A classic example of primary successional development is the sand dune ecosystem, where youngest stages of primary succession are characterized by non-mycorrhizal plant species, followed by facultative and obligate AM plant species, and later by ECM and ERM plant species along succession (Read, 1989). This example is not the case in all successional trajectories, and divergent successional changes in mycorrhizal communities reflecting ecosystem properties have been reported (e.g. Zangaro, Alves, Lescano, Ansanelo, & Nogueira, 2012; Zemunik, Turner, Lambers, & Laliberté, 2015). In addition to mycorrhizal fungi, the presence of other RAF varies during succession (Poosakkannu*,* Nissinen, & Kytöviita, 2015; Tejesvi Ruotsalainen, Markkola, & Pirttilä, 2010). Interconnected successions of RAF and plant communities show the importance of these interactions (Clemmensen et al., 2015; Teste & Dickie, 2016). RAF have relevant roles in nutrient (Bödeker et al., 2014; Grelet et al., 2016; Phillips et al., 2014; Read & Perez-Moreno, 2003) and carbon cycling (Clemmensen et al., 2013; Klein et al., 2016; Soudzilovskaia, Vaessen, van't Zelfde, & Raes, 2017; Subke, Voke, Leronni, Garnett, & Ineson, 2011) in boreal and subarctic forests. As ECM fungi form a route for recently fixed C from trees to soil, they also carry a priming effect to degradation of older C in SOM (Subke et al., 2011) having an impact on succession. Furthermore, diversity of plant and fungal communities are connected. Diversity of mycorrhizal symbionts and nutrient acquisition strategies have been shown to relate to increased diversity of host plant species (Teste et al., 2017; van der Heijden, Bardgett, & van Straalen, 2008). Higher diversity often links with higher resilience of ecosystem functions (Oliver et al., 2015).

Disturbances have impacts on species diversity of RAF and they may shift the community composition from one state to another (e.g. Saravesi et al., 2015; Treu et al., 2014). In general, species coexistence depends on the competition / colonization trade-off. According to the intermediate disturbance hypothesis (IDH), disturbance interrupts the process of succession, so that good colonizers are favoured at high levels, good competitors at low levels and at intermediate levels of disturbance coexistence of both types of species is promoted. In the patchy environment, species coexistence is the highest at the intermediate levels of disturbances (Connell, 1978; Grime, 1973; for a review see Shea, Roxburgh, & Rauschert, 2004). In theory, the highest diversity should occur also at the intermediate levels of succession, when neither good colonizers nor competitors dominate (Connell, 1978; Grime, 1973; Shea et al., 2004). As successional drivers (Meiners, Cadotte, et al., 2015; Teste & Dickie, 2016) also species diversity patterns seem to be scale dependent (Bahram, Peay, & Tedersoo, 2015; Tedersoo et al., 2014; Willis & Whittaker, 2002).

New research methods using molecular tools, have enabled more detailed studies of belowground fungal diversity (Shokralla et al., 2012; Tedersoo et al., 2014). In this thesis, conventional and more recent methods (next-generation sequencing, NGS) are applied to study RAF in grass and tree roots, with an emphasis on the responses of the fungal communities to disturbance.

1.4 Aims of the study

In this thesis, the responses of root-associated fungal (RAF, incl. mycorrhizal fungi) communities to natural and anthropogenic disturbances in boreal and subarctic forests are investigated. Along succession and after disturbance, site conditions (e.g. soil pH, nutrient resources, carbon cycling), species availability (e.g. vegetation, RAF inoculum potential) and species performance (e.g. species interactions) may change. In the following three studies, the main interest was in changes of plant-fungal interactions and their relation to the other types of changes in ecosystem.

This thesis addresses the following questions. In the case of the first study question, specific hypotheses are formed.

1. Do RAF community composition and diversity change after disturbance? What kind of changes are these? Do impacts of disturbance differ between disturbance intensity levels? (Papers I, II, III)
2. Do changes in RAF co-occur with changes in soil environment and in vegetation? (Papers I, II, III)

In the first study (I), impacts of removal of a dominant neighbouring plant on RAF (AM and DSE) colonization of wavy-hair grass were studied along a primary succession gradient. Aboveground parts of the neighbouring mountain crowberry were clipped once in the summer. It was expected that this would change interactions between the plants and affect their RAF (Bever et al., 2010). Roots of both plant species are colonized by DSE fungi (Tejesvi et al., 2010). Abiotic disturbance decreases from the young successional stages in an inland direction to older successional stages. Furthermore, soil characteristics, plant-plant and plant-fungal interactions are known to change along succession (Smith & Read, 2008). The specific hypotheses are, that

> H1 a) removal of the aboveground parts of the neighbouring plant would alter DSE colonization of wavy-hair grass, and that
>
> H1 b) root fungal colonization types (AM, DSE) would change along succession.

In the two other studies (II, III), the main interest was in how RAF communities of transplanted tree seedlings change after intensive disturbances. This point of view was taken, because the first years of seedling growth are crucial to seedling survival, and thus forest regeneration. Characteristics of the disturbances in these two studies differed from each other. Defoliation in the second study (II) has more direct impacts on aboveground vegetation than on soil, whereas in the third study (III), clear-cutting and soil modification disturb both above- and belowground parts of the ecosystem heavily.

In the second study (II), impacts of moth herbivory on soil fungal communities, and further, on the formation of RAF communities of transplanted mountain birch seedlings were studied. The study included two parallel experiments, in which the treatments were directed to the adult mountain birch and understory vegetation in each plot, whereas transplanted seedlings were kept intact. In the first experiment, the aim was to study the effects of natural moth feeding, and in the second, to separate the impacts of defoliation and moth frass fall using simulations. Previous studies have shown that natural moth herbivory decreases abundance of ECM fungi, whereas abundance of more opportunistic and saprotrophic fungi in the roots may increase with increasing years of moth herbivory (Saravesi et al., 2015). Accordingly, also simulated defoliation can decrease carbon flow from the defoliated host plant to ECM fungi, whereas moth fertilization can decrease the nutrient acquisition-related benefits that the host plant may gain from ECM fungi (Smith & Read, 2008). Changes in carbon flow from the trees to the soil following defoliation and additional nutrient inputs may affect also soil fungal communities. The specific hypotheses are the following:

> H2 a) natural moth feeding would decrease abundance of ECM fungi in transplanted mountain birch seedling roots and in soil (abundance of ECM fungi being lower and abundance of more opportunistic fungi being higher in a three-year moth feeding compared to control and a two-year treatment),

> H2 b) simulated defoliation and fertilization with moth frass would change RAF community composition of mountain birch seedlings compared to the control, and that
>
> H2 c) defoliation and additional nutrient inputs as moth frass would affect soil fungal community composition in both experiments.

In the third study (III), impacts of clear-cutting and different intensity levels of energy wood harvesting practices on RAF communities of Norway spruce seedlings were studied. Because energy wood harvesting increases disturbance, it may affect ECM inoculum in soil and thus RAF communities in Norway spruce seedling roots. I hypothesized, that

> H3) energy wood harvesting would result in lower ECM fungal diversity in Norway spruce seedling roots, and RAF community composition would change along with increasing complexity of energy wood harvesting practices.

In the first study (I), root fungal colonizations were studied microscopically and in the second (II) and the third (III), RAF communities were identified using NGS. Impacts of disturbances on diversity of different fungal guilds were estimated (II, III). In all studies, impacts on soil nutrients were measured. In the second study (II) also soil ergosterol was examined. Also, host plant-, field layer vegetation- and canopy-related parameters were recorded in all studies and their relation to changes in RAF studied.

2 Material and methods

Responses of RAF communities to disturbances were tested in three experiments. RAF community compositions were studied microscopically (I) or with NGS (II, III). Different plant host species were used as study species to formulate a more comprehensive view of RAF dynamics. In the following parts of this work, I refer to the different studies, in addition to the Roman numerals (I, II, III), by the names given according to the genus of the study species (*Deschampsia*, *Betula* and *Picea* study, respectively).

2.1 Study species

Wavy-hair grass (*Deschampsia flexuosa* (L.) Trin) is a common boreal understory species that thrives in many different environments, being especially abundant in disturbed ones. For example, it grows on the sandy coasts of the Bothnian Bay, boreal forests and subarctic heaths (Hämet-Ahti, Suominen, Ulvinen, & Uotila, 1998). *D. flexuosa* shows relatively high morphological and physiological plasticity and tolerance to multiple disturbances (Hedwall, Brunet, Nordin, & Bergh, 2013; Oksanen & Ranta, 1992; Ruotsalainen et al., 2007; Strengbom, Walheim, Näsholm, & Ericson, 2003). *D. flexuosa* forms arbuscular mycorrhiza with Glomeromycota and its roots also associate with endophytic fungi mainly belonging to Helotiales (Ruotsalainen et al., 2007; Tejesvi et al., 2010).

Mountain crowberry (*Empetrum nigrum* ssp. *hermaphroditum*) is an evergreen dwarf shrub, which grows in subarctic and circumpolar areas (Hämet-Ahti et al., 1998). It forms dense mats of nearly monospecific vegetation on acidic and nutrient-poor soils when disturbances are infrequent and the climate is cool (Tybirk et al., 2000). It can produce allelopathic compounds through which it negatively affects the growth of other plants (Nilsson, 1994; Zackrisson & Nilsson, 1992). Crowberry dominates in numerous ecosystems, e.g. heathlands and forests (Tybirk et al., 2000). These ecosystems may be associated with humus build up, because the roots and other parts of this understory species decompose slowly (Clemmensen et al., 2015; Wardle, Zackrisson, Hörnberg, & Gallet, 1997). Mountain crowberry forms ericoid mycorrhiza with ERM fungi (Tybirk et al., 2000) and its roots also associate with endophytic fungi (Kjøller, Olsrud, & Michelsen, 2010; Ruotsalainen Markkola, & Kozlov, 2010; Tejesvi et al., 2010). Crowberry was not studied as a focal plant host, but it was a dominant neighbouring species in the *Deschampsia* study and a dominant understory species in the *Betula* study.

Mountain birch (*Betula pubescens* ssp. *czerepanovii* (Orlova (Hämet-Ahti)), formerly called *B. p.* ssp. *tortuosa*), is a dominant altitudinal and latitudinal treeline species in the subarctic zone in Fennoscandia. It is a small (1–6 m tall), deciduous, monoecious, strongly self-incompatible, wind-pollinated, sometimes shrub-like tree. The mountain birch trunk is typically curved and polycormic. Mountain birch shows a tendency towards vegetative propagation (Atkinson, 1992). The first growing seasons may be critical for mountain birch survival (Lehtonen & Heikkinen, 1995; Sveinbjörnsson, Kauhanen, & Nordell, 1996).

Norway spruce (*Picea abies* (L.) H. Karsten) is a common boreal coniferous tree species. It is a monoecious, wind-pollinated and shade-tolerant species. As a successful competitor, it forms first a lower canopy layer beneath taller trees, and eventually overgrows them (Hämet-Ahti et al., 1998). Norway spruce is a typical tree species in late successional forests. Norway spruce-dominated forests comprise 30% of cultivated forests in Finland (Natural Resources Institute Finland, 2017). Both tree species (mountain birch and Norway spruce) grow in symbiosis with multiple ECM fungi, and are dependent on their partners in nutrient acquisition (Smith & Read, 2008).

2.2 Experimental designs

Responses of RAF to succession and disturbance were studied in three field experiments during years 2008–2013. The duration of each study was 2–4 years. The *Deschampsia* study (I) was conducted in the middle boreal, the *Betula* study (II) in the subalpine mountain birch zone at the northern boundary of the northern boreal, and the *Picea* study (III) in southern and middle boreal vegetation zones (Ahti, Hämet-Ahti, & Jalas, 1968).

Deschampsia study (I)

A field experiment was conducted to understand how aboveground disturbance alters plant-plant and plant-fungal interactions and how succession affects root fungal colonization of wavy-hair grass, *D. flexuosa.* The study was performed on a land-uplift coast of the island of Hailuoto (65°02'N, 24°35'E) in the Gulf of Bothnia. As a consequence of post-glacial land-uplift, Hailuoto still emerges from the sea at an annual rate of 8.6–9.0 mm (Alestalo, 1979) enabling the study of successional changes in RAF. The soil of the study area is sandy and nutrient-poor with a low pH (Grau et al., 2010; Pennanen, Strömmer, Markkola, & Fritze, 2001). The forests

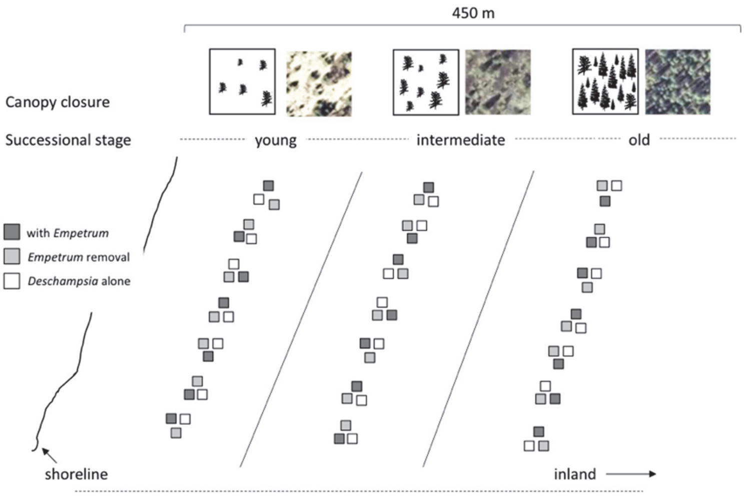


Fig. 1. Experimental set-up in the *Deschampsia* study (I). The figure illustrates one of the three successional transects from the shore to inland. Each transect was divided into three successional stages (young, intermediate, old) according to vegetation and canopy closure. On each stage, seven blocks consisting of three experimental treatment plots (with *Empetrum*, *Empetrum* removal, *Deschampsia* alone) were established.

of the island are dominated by Scots pine (*Pinus sylvestris* L.). Vegetation changes with increasing successional age and soil pH decreases from pH 6 at the dune ridge to pH 4.3 in the forest (Grau et al., 2010). Three transects from shoreline to inland (Marjaniemi, Mäntyniemi, Virpiniemi) were divided in three successional stages according to their vegetation cover and distance from the shoreline: young, intermediate and old (shore dune backs, deflation basin, mature forest, respectively, Fig. 1). At each site, seven replicate blocks for each successional stage were established in early June 2009. One block consisted of three 0.25 m^2 plots, which were randomized within each block. To control the impacts of the neighbour, for one of three plots, lone *D. flexuosa* plants without *E. nigrum* ssp. *hermaphroditum* close by were selected (the distance to *E. nigrum* ssp. *hermaphroditum* more than 0.4 m, treatment level = *Deschampsia* alone). In two other plots, *D. flexuosa* were accompanied with *E. nigrum* ssp. *hermaphroditum* as the dominant neighbour. Interaction between the plant species were modified by disturbing the growth of neighbouring dwarf shrub. In a randomly selected plot from each plot pair, living aboveground parts of *E. nigrum* ssp. *hermaphroditum* were cut off at ground level with scissors (treatment level = *Empetrum* removal) and not from the other plot (treatment level = with *Empetrum*) (Fig. 1). The treatment was repeated the following year, when new sprouts of *E. nigrum* ssp. *hermaphroditum* were cut off. Cut biomass was removed from the plots. The experimental setup included altogether three transects, 63 blocks and 189 plots, totalling 189 *D. flexuosa* individuals.

Betula study (II)

In order to study impacts of moth herbivory on RAF and soil fungi in the subarctic mountain birch forest, two parallel experiments were conducted. In total 49 experimental plots of 1 m^2 were established in natural mountain birch forest on a northern slope of Mt. Buksalskáidi in 2011. The plots were arranged into seven blocks each approximately 200 m^2 in size. Each study plot had a mountain birch tree (1–1.5 m in height) in the plot centre. Root connections to surrounding vegetation were cut by trenching, up to mineral soil layer to the depth of 15–20 cm, conducted repetitively at the beginning of each growing season in late May. The total number of experimental plots was 21 in the natural moth feeding experiment and 28 in the simulated moth feeding experiment.

To mimic a natural moth outbreak, a feeding experiment on mountain birch with autumnal moth (*Epirrita autumnata* (Borkh.)) larvae and winter moth (*Operophtera brumata* (L.)) larvae was set up. The experiment was carried out in 1 x 1 x 1 m cages made of thin 100% polyester fabric (mesh size 0.109 mm^2) and wooden frames. Cages were set in the beginning and removed in the end of each growing season (i.e. end of May – mid-September). The experiment included three levels of moth larvae feeding treatment. These were control (cage without moth larvae; control), moth feeding on mountain birch during two years (in 2012–2013; 2-yr), and moth feeding on mountain birch during three years (2011–2013; 3-yr) (Fig. 2). All the cages were set in 2011, but the feeding in cages of the two-year treatment was started only in 2012. Moth larvae were released to cages in four sets: once in a week in during a four-week period starting from the beginning of growing season in early June in each year. Similar sets of larvae were inserted to each experimental plot at given time, but developmental stages and number of larvae varied between addition times.

Parallel to the moth feeding experiment, a simulated moth herbivory experiment with defoliation of a mountain birch tree and shoot damaging of understory vegetation (defoliation and damaging, D), moth frass addition as a fertilizer treatment (moth frass, F) and with their interaction (DF) was set up (Fig.

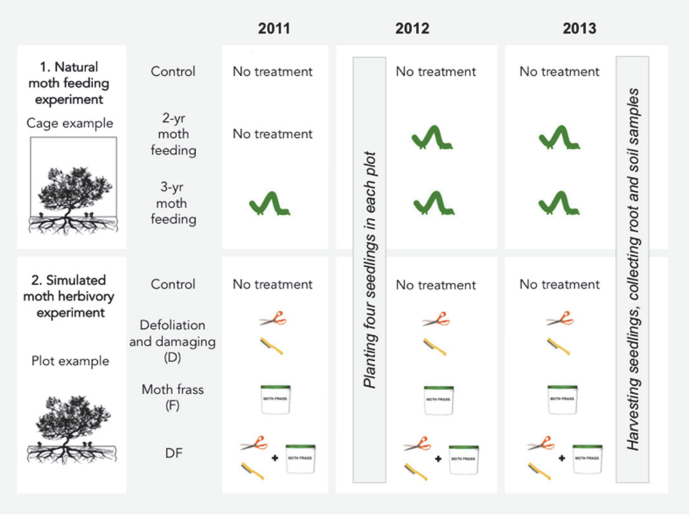


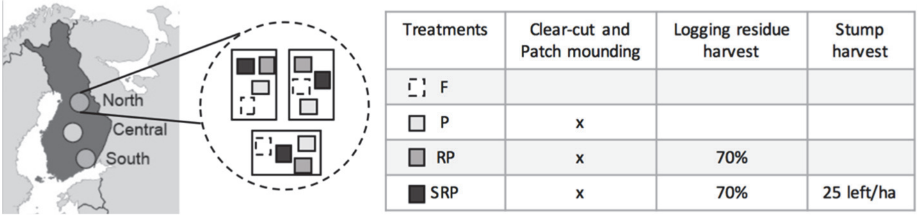
Fig. 2. Experimental set-up in the *Betula* study (II). The figure illustrates experimental treatments during 2011–2013. The study consists of two parallel experiments. The natural moth feeding experiment included a Control without moth larvae, and two-year (2-yr) and three-year (3-yr) moth feeding treatments. The simulated moth herbivory experiment included a Control plot, Defoliation and damaging treatment (D), Moth frass addition (F) and a combination of two previous treatments (DF). Seedlings were planted in the plots in June 2012 and harvested in September 2013. The treatments were replicated in seven blocks.

2). Treatments were applied during 2011–2013. Experimental plots were set up in seven blocks in full-factorial design. To simulate moth folivory in D and DF plots, the leaves of the mountain birch were removed with scissors and the understory dwarf shrubs were damaged using a metal wire brush and a plastic cutting board to mimic the non-systematic way larvae feed on dwarf shrubs. To mimic fertilization effect resulting from moth outbreak, an average of 163 ml of dried moth frass (corresponding approximately frass production of 200-250 *E. autumnata* moth larvae during larval development period) was added with 1200 ml of water to F and DF plots once during each growing season. Water, but no moth frass, was added to D and Control plots. F was carried out simultaneously with defoliation and damaging treatment (D) after leaves were fully grown in late June in each year of the experiment.

Four three-month-old mountain birch seedlings of local origin were planted as pairs at a distance of 30 cm from the stem of the established mountain birch tree in each experimental plot in July 2012. In the moth feeding experiment, seedlings were covered with shields made of polyamide fabric and wire during the period of moth feeding to avoid unwanted damage to the seedlings by moths. Seedlings were harvested after two growing seasons in autumn 2013, and the RAF communities were studied with NGS.

Picea study (III)

In order to test the short-term impacts of clear-cutting and energy wood harvesting on RAF communities of Norway spruce seedlings, the field experiment was conducted in Norway spruce-dominated sites in three different geographical areas in Finland (Fig. 3). Within each of three study areas, three replicate blocks consisting of four experimental plots of ca. 40 m × 40 m were established. The blocks of 0.5–1 ha were located at a distance ranging from 0.2–15 km from each other. Three of the four were experimental clear-cut plots with patch mounding and different energy-wood harvesting treatments increasing in complexity: 1) patch mounding alone (P) to 2) patch mounding with 70% of logging residue removal (RP), and 3) patch mounding combined with both 70% of logging residue removal and partial stump removal (SRP) with 25 stumps left per hectare. The last one of four plots was the uncut reference plot (F). The total number of experimental plots was 36 (i.e. 3 geographical areas × 3 replicate blocks × 4 treatment plots, Fig. 3).



Treatments	Clear-cut and Patch mounding	Logging residue harvest	Stump harvest
⬚ F			
☐ P	x		
▨ RP	x	70%	
■ SRP	x	70%	25 left/ha

Fig. 3. Experimental set-up in the *Picea* study (III). The study was conducted in three different geographical areas (South, Central and North) in Finland. Within each area, three blocks were established. Each block consists of four plots: three of these (P, RP, SRP) were situated at clear-cuts and one in uncut forest (F). At clear-cut plots, impacts of increasing complexity of energy wood harvesting practices on RAF of transplanted Norway spruce seedlings were studied. The patch mounding (P) treatment included clear-cutting and patch mounding, but no energy wood harvesting practices. The logging residue harvesting (RP) treatment included actions of P and additional 70% logging residue harvesting. The stump removal (SRP) treatment included actions of RP and additional stump harvesting so that no more than 25 stumps / ha were left on the site. Norway spruce seedlings were planted on the sites in 2008 and harvested in 2011.

Trees were felled from all experimental plots during winter 2007/2008 and delimbed at the plots. Logging residues (branches with needles) were collected with a bundler, and stumps were dug out of the ground with an excavator in June 2008 according to harvesting treatments and left at the plots for six weeks to dry before removing. In the harvesting treatment P, where logging residues were not collected, they were spread evenly on plots. In August 2008, 1.5-year-old nursery-grown Norway spruce seedlings were planted on four mounds (the height 0.2 m, approx. 0.6 m^2 in the size) located in different quadrants of each harvesting treatment plot and in a similar fashion on intact forest floor in reference forest plots at all experimental areas. The distance between planting mounds was 10–20 m. Seedlings of local origin were used in all experimental sites. Stand properties (e.g. age, density and height) and climatic factors varied depending on the area. The experiment was set up by the Finnish Forest Research Institute (METLA, the present Natural Resources Institute Finland (LUKE)) and experimental sites owned by UPM Kymmene.

2.3 Field sampling and laboratory analyses

Plant material and parameters

Study plants were harvested after two (I, II) or three (III) full growing seasons. Plants were stored at 6°C (I, III) or -20°C (II) until processed further. Shoots were separated from roots, and current-year shoot height growth (III) or shoot height was measured (II), and shoot dry weight was determined after drying plants 48 h at 40°C (II, III). In study I, green foliar biomass for measuring foliar C and N was collected from 154 study plants, and dried at 70°C for 48 hours. Samples were homogenized and foliar C and N concentration analysed using an automatic CHN analyser (Fisons Instruments, Milan, Italy).

The root system was manually separated from soil and gently washed in a container filled with tap water. Roots samples were collected systematically around the whole plant root system (III) or all the roots of the plant were sampled (I, II). Root samples were further cleaned under a stereomicroscope using forceps and a random subset of fine roots (<1 mm) (II, III) or all the roots (I) were collected, and stored at -20 °C (II, III) or in 50% ethanol (I). In the *Betula* (II) and *Picea* (III) studies, fine roots were freeze dried for 24 h in -48°C and their dry weight was determined before DNA extraction.

Soil parameters

Soil parameters were determined in all studies to estimate their impacts on RAF communities. Soil sample numbers and methods for nutrient analyses used in each study are presented in Table 2.

In the *Deschampsia* study (I), soil samples were collected with a soil borer (3 cm in diameter) from humus and mineral soil layers. All organic soil, but only five uppermost centimetres of mineral soil, were taken from each core. At the youngest successional stages the humus layer had not yet formed. If a humus layer existed, soil cores were divided to organic and mineral soils and put into their own bags. One sample consisted of eight cores of soil. Five samples were collected from each site and layer.

Table 2. Information about soil analyses in studies I, II, and III. Abbreviations: - = not determined, LOI = loss of ignition, AAS = atomic absorption spectrometer.

	Deschampsia study (I)	*Betula* study (II)	*Picea* study (III)
Size of one soil sample	eight 3 cm soil cores	five vertically halved 3 cm soil cores	12 to 16 spoonful's (15 ml)
Sample number (soil type)	45 (mineral) 30 (organic)	49 (organic)	36 (mixed, near the roots)
Humus thickness	determined	determined	-
Storing	one week in 4°C	in -20°C	in -20°C
Removal of rocks and roots	yes	yes	yes
Sieving	no	yes	no
Moisture content	-	-	105 °C, 20 h
LOI	550°C, 3.5 h	550°C, 3.5 h	500°C, 4 h
pH	water extracts	water extracts	-
Electrical conductivity	water extracts	water extracts	-
Total C	ISO 16948:2015	automatic CHN analyzer	-
Total N	ISO 16948:2015	automatic CHN analyzer	modified Kjeldahl method
Ammonium N	-	colorimetric method	-
Soluble P	-	ammonium acetate extracts, colorimetric method	modified molybdenum blue method
Soluble cations (Ca, K, Mg)	-	ammonium acetate extracts, AAS	-
Soil ergosterol	-	modified ergosterol assay	-

In the *Betula* study (II), soil samples were collected with a soil borer (3 cm in diameter) from humus layer. Five soil cores collected from the same experimental plot were divided vertically into two equal halves resulting two parallel pooled organic soil samples per a plot. The first sample was used for nutrient analyses and the second for DNA extraction and ergosterol analysis. Soil ergosterol was analysed to obtain estimates for soil fungal biomass. Ergosterol was extracted using a modified ergosterol assay (Nylund & Wallander, 1992) from freeze-dried, ground soil samples of 100 mg DW. Extracts were quantified with high-pressure liquid chromatography (HPLC) using a reverse-phase C18 column equipped with a precartridge and methanol as the eluent (1.0 mL min^{-1}, column temperature +30 °C). Commercial ergosterol (5,7,22-Ergostatrien-3ß-ol, Fluka AG) was used as a standard.

In the *Picea* study (III), soil samples were taken from the soil around the roots. Four random soil samples of ca. 15 ml were taken with spoon from the vicinity of each seedling's roots. Soil samples from the same plot were pooled forming at total of 36 soil samples. In harvesting treatments (P, RP, SRP) the samples consisted mostly of mineral soil from mounds and in the forest (F) the samples included soil from organic and mineral layers.

Fungal colonization and community composition

Roots of *D. flexuosa* plants were stained with trypan blue (method by Phillips and Hayman (1970) modified as given in Tejesvi, Sauvola, Pirttilä, and Ruotsalainen (2013)), and their fungal colonization was estimated according to the magnified intersection method (McGonigle, Miller, Evans, Fairchild, & Swan, 1990) in the *Deschampsia* study (I). AM colonization was measured as hyphal, arbuscular and vesicular colonization and DSE colonization as hyphal, sclerotial and hyaline septate endophyte-type hyphae (HSE, see Barrow & Aaltonen, 2001; Yu et al., 2001). About 20 cm of root material with 100 intersects were studied in a sample.

In *Betula* and *Picea* studies, fungal community compositions were determined by next-generation sequencing (NGS) methods. First, freeze-dried samples were homogenized for DNA extraction. Root samples were pulverized either in Eppendorf tubes by cooling the TissueLyser adapter set and tubes with liquid nitrogen before grinding with a TissueLyser (Qiagen) (II), or with a pestle and liquid nitrogen in a mortar (III). Organic soil samples were homogenized in liquid nitrogen in a mortar (II). Secondly, samples from the same study plot were pooled by combining all root or soil samples from the same plot before grinding (II) or by taking 35–40 mg DW of each homogenized root sample (III). DNA was extracted from 20 mg DW of pulverized root (II, III) or 100 mg DW of ground organic soil using a MoBio Power Soil® DNA isolation kit (II). DNA originating from roots was eluted in 50 ml (II, III) and soil DNA in 100 ml of the elution buffer C6 (II). All templates were quantified with a Nano-Drop ND-1000 UV-Vis Spectrophotometer (Thermo Scientific, Wilmington, DE) and adjusted to a final 5 ng µl-1 for PCR amplification (II, III). The Internal Transcribed Spacer 2 (ITS2) region was amplified for sequencing using fungal specific primer sets ITS4 (White, Bruns, Lee, & Taylor, 1990) and fITS7 (Ihrmark et al., 2012) (II) or ITS4 (White et al., 1990) and 58A2F (Martin & Rygiewicz, 2005) (III). In the both studies, a 10 bp unique tag was added in the ITS4 primer. The fungal ITS2 region was amplified in 20 µl volumes in three separate PCR reactions to account for potentially heterogeneous amplification from the root template (II, III). Amplified PCR products from three reactions were combined totalling the volume of 60 µl. DNA extraction, PCR amplifications, and purification of PCR products for sequencing are described in detail in papers II and III. Amplicons were sequenced with an Ion Torrent^{TM} Personal Genome Machine® (PGM) (II) or with a 454 Roche GS Junior (III) at Bioser Oulu, Department of Biology (currently the laboratory belongs to Biocenter Oulu), University of Oulu. In *Betula* study, sequencing was conducted in two Ion Torrent runs, each in 314 chip and 400 bp chemistry, due to a large sample number. The first run included amplicons for root samples and the second amplicons for soil samples.

Sequences were divided to samples (III), quality-filtered and clustered to operational taxonomic units (OTUs) (II, III) using the Quantitative Insights into Microbial Ecology (QIIME) (Caporaso et al., 2010). In study II, mothur (version 1.31.2; Schloss et al., 2009) and cutadapt (Martin, 2011) were used for dividing the sequences to samples, removing primers and for quality filtering. In the both studies, the aim was to filter out the low-quality sequences and to remove ambiguous bases, primer mismatches, and sequences with length <100 bp (II) or <150 bp (III) or >1000 bp (II) or >500 bp (III) or those missing the primer from the other untagged end (II). In the *Picea* study (III), sequencing noise was removed by flowgram clustering with Denoiser (Reeder & Knight, 2010). OTUPIPE (Edgar, Haas, Clemente, Quince, & Knight, 2011) (II) and UCLUST (Edgar, 2010) (II, III) were used for OTU clustering. In the *Betula* study (II), chimeric sequences were removed using UCHIME (Edgar et al., 2011) using a curated data set of fungal ITS sequences (Nilsson et al., 2015) as reference data. Representative sequences were queried against UNITE (Kõljalg et al., 2013) and NCBI databases using BLAST tools implemented in the PlutoF workbench (Abarenkov et al., 2010). BLAST results were examined carefully, especially the graphical overview and pairwise alignments, to detect anomalies and chimeras. Non-fungal OTUs, chimeric OTUs, or OTUs whose query coverage was < 85% (II) or < 80% (III), and thus considered to be unreliable were removed. The taxonomically most accurate hit was selected with ≥ 97% sequence similarity. Fungal guilds were determined according to taxonomic assignments. Bioinformatics and sequence annotation and naming are described in detail in papers II and III. In the *Betula* study, the FUNGuild tool (Nguyen et al., 2016) was used for a primary functional analysis of fungal OTUs. Fungal guilds used in the *Betula* and *Picea* studies differed from each other. In both studies classifications were formed for ECM (II, III), ERM and endophytic fungi within RAF (II) or other mycorrhizal (III), saprotroph/pathogens (II, III), lichens (II), other fungi (III), and undefined (II) / functionally unknown (III) fungi. Diversity indices (rarefied richness (II), Shannon's H' (II, III), Simpson's D (II), inverse Simpson's D (III)), based on occurrences and abundances of fungal OTUs, were calculated with the vegan package (Oksanen et al., 2013, 2017) using R (R Development Core Team, 2013, 2016).

2.4 Statistical analyses

All statistical analyses were carried out using R and its versions 2.15.3 (III) (R Development Core Team, 2013), 3.2.4 (I) (R Development Core Team, 2015) and 3.3.2 (II) (R Development Core Team, 2016). Differences in root fungal colonizations (I), sequence abundances and OTU proportions of fungal taxonomic groups and fungal guilds (II, III), diversity indices (II, III), and other background data (II) were analyzed using linear mixed models with the package nlme (I, II) (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2016) or lme4 (III) (Bates, Maechler, & Bolker, 2011). In the *Picea* study (III), count data were analyzed using a generalized linear mixed model (glmer, Laplace approximation) with Poisson error distribution, and continuous data by a linear mixed model (lmer, restricted maximum likelihood) with Gaussian error distribution.

In the *Deschampsia* study, successional stage (levels = young, intermediate, old) and *Empetrum* removal treatment (levels = *Empetrum* removal, with *Empetrum*, *Deschampsia* alone) were used as fixed factors, and site, successional stage within site and block within site and successional stage as random factors. In the *Betula* study, treatment (Control, 2-yr, 3-yr) was used as a fixed factor (n=3 levels) and block as a random factor (n=7) to study effects of moth feeding. In the case of the simulated moth herbivory experiment, the lme model with moth frass addition (F, yes vs. no) and damage (D, yes vs. no), and their interaction as a fixed factors and block (n=7 for roots, n=6 for soils) as a random factor was applied. In the *Picea* study, 'treatment' (SRP, RP, P and F) was used as a fixed factor and 'geographical area' (South, Central and North) and 'block within area' were used as random factors. For differences between the geographical areas within each treatment, 'area' was used as a fixed factor and 'block' as a random factor. Arcsine-square root transformations were used to normalize the distribution and homogenize the variation of residuals of proportion data (I, II, III) (Crawley, 2007). Normality and homogeneity of residuals were checked using Q–Q and scatter plots (I, II, III). If ANOVA F test results were statistically significant ($p < 0.05$), Tukey HSD tests were conducted on all possible pairwise contrasts with the "glht" command using the "multcomp" package (Hothorn, Bretz, & Westfall, 2008) in R (I, II). In the *Picea* study, the estimated difference between two means was considered as statistically significant ($p < 0.05$), when the absolute value of t- or z-score in mixed models was ≥2 (Crawley, 2007). The t- and z-scores were obtained by dividing the model parameter estimates (coefficients) by model SE. In the case of statistically significant results, pairwise comparisons were carried out.

Fungal OTU assemblages of roots (II, III) and soil (II) were visualized using non-metric multidimensional scaling (NMDS) and compared using permutational multivariate analysis of variance in the vegan package (adonis, Oksanen et al., 2013, 2017) (II, III). NMDS ordinations and tests were based on the presence–absence data and Jaccard indices (III) or also Hellinger-transformed abundance data and Bray-Curtis dissimilarity indices (II, III). The function envfit from the vegan package (Oksanen et al., 2013, 2017) was used to identify variables that were significantly correlated with community composition of RAF (II, III) and soil fungi (II). The significance of correlations was assessed by comparing the r^2-fit to r^2-values generated via 999 random permutations of these variables. Relationships between fungal OTUs and different treatments were detected by indicator species analysis with the indicspecies package (De Cáceres & Legendre, 2009) (III).

The relationships between the characteristics of RAF communities and the foliar carbon concentration of the host plant (I) or soil ergosterol (II) were studied using scatterplots and by calculating Pearson correlation coefficients. The relationships between AM and DSE hyphal colonization were studied similarly (I).

3 Results and discussion

Disturbance altered RAF communities depending on its target, intensity and scale (Question 1). The impacts were more evident, when either the disturbance affected soil and surrounding vegetation on a wide scale, or neighbouring plant being same species with the study plant, and, moreover, when the study plants were still in juvenile phase (natural moth feeding in the *Betula* study (II), clear-cutting and patch mounding in the *Picea* study (III)). Aboveground disturbance altering neighbouring plant, that was different species, had no impacts on the RAF of the adult study plant (damaging of *Empetrum* in the *Deschampsia* study (I)). Results implied that wider-scale disturbance or other environmental changes (e.g. land-uplift succession (I), clear-cutting and patch mounding (III)) may mask the impacts of less intense disturbances (e.g. damaging of neighbouring plant (I), energy-wood harvesting (III)). Changes in RAF communities co-occurred with the changes in soil (I, III) and in vegetation (I, II, III) (Question 2). The results of the thesis are summarised in Table 3, and discussed in detail below.

3.1 Impacts of disturbance on RAF community composition and diversity

In the *Deschampsia* study (I), no connection between disturbance affecting neighbouring plant and RAF of the study plant was found, while a shift in RAF was related with successional changes in the environment. In the *Betula* and *Picea* studies (II, III), disturbance altering soil and vegetation changed RAF community composition and RAF diversity of tree seedlings. RAF community composition was also affected by the intensity of disturbance in the *Betula* study (II).

RAF community composition

In the *Deschampsia* study (I), fungal colonization of wavy-hair grass roots was not affected by the removal of aboveground parts of neighbouring plant (contrary to Hypothesis 1a), even though this should, at least in theory, change interactions between neighbouring plant species (Bever et al., 2010). Instead, RAF colonization type of wavy-hair grass changed along with increasing successional age and with a decrease in potential sources of disturbance in the environment (Hypothesis 1b). AM fungi dominated in the roots of wavy-hair grass at young successional stages, whereas DSE fungi were more common in the roots at older successional stages

Table 3. Disturbance type, study plants and affected root-associated fungal (RAF) groups, and detected responses to disturbance in each study. Abbreviations: AM = arbuscular mycorrhizal fungi; ECM = ectomycorrhizal fungi; ERM = ericoid mycorrhizal fungi; DSE = dark septate endophytes; D = simulated defoliation and damaging treatment; F= moth frass addition; EWH= energy-wood harvesting; CC = clear-cutting and soil modification; ↑= increase; ↓= decrease.

	Deschampsia study (I)	*Betula* study (II)	*Picea* study (III)
Disturbance	Damaging of neighbouring plant Primary succession	Natural moth feeding on adult birch Simulated defoliation (D) on adult birch Moth frass addition (F)	Energy-wood harvesting (EWH) Clear-cutting with soil modification (CC)
Study plant	Wavy hair grass	Mountain birch seedling	Norway spruce seedling
Shifts in RAF group	Roots: AM, DSE	Roots: ECM, *Meliniomyces*, DSE; Soil: ERM	Roots: ECM
Question 1 – Do RAF community composition and diversity change after disturbance? Do impacts of disturbance differ between intensity / frequency levels?			
Impacts on RAF community composition	Damaging of neighbouring plant had no impact on root colonization; A shift from AM to DSE dominated along succession	Shifts between ECM and DSE and *Meliniomyces* in seedling roots after natural moth feeding; A shift in the abundance of ERM after F in soil	EWH did not have clear impacts in the short term; *Thelephora* dominated RAF of seedling after CC
Impacts on RAF diversity	Not studied	Changes in diversity and dominance	Diversity higher in uncut forest
Differences between intensity / frequency levels of disturbance	AM fungi dominated at young successional stages, DSE fungi at old successional stages	*Meliniomyces* and DSE most abundant and diversity the highest in 2-yr moth feeding, ECM most abundant in 3-yr	No clear differences between different intensity classes of EWH in the short term (masked by CC)
Question 2 – Do changes in RAF co-occur with changes in soil environment and in vegetation?			
Soil environment	Soluble P↑, pH↓along succession	Generally small changes	EWH: P↑, forest: SOM%↑, N in OM↑
Vegetation	Dwarf shrubs↑, canopy closure↑	Host birch↓, compensatory↑in *Empetrum*	CC in EWH treatments, host spruce↓

(Fig. 1 in I). On a succession gradient, disturbance formed by abiotic factors (e.g. wind, unstable soil) is the highest at young successional stages. However, impacts of these factors on complex species interactions are difficult to estimate, partially due to ecological differences between species. According to the results of the *Deschampsia* study, small-scale changes in individual plant-plant interactions have relatively slight impacts on root fungal colonization types, the patterns of which seemed to be governed by large-scale successional changes in the environment (discussed more in section 3.2.).

In the *Betula* study (II), RAF community composition was affected by natural moth feeding (Hypothesis 2a; Fig. 3, Table 3 and Supplement S4 in II). Contrary to Hypothesis 2a, abundance of ECM fungi in mountain birch seedling roots was not the lowest in the longest moth feeding treatment (3-yr moth feeding), but in the shorter moth feeding treatment (2-yr moth feeding). In the two-year moth feeding treatment, also abundances of *Meliniomyces* and *Phialocephala* were higher compared to the control and the three-year moth feeding treatment. Ecological roles of *Meliniomyces* and *Phialocephala* are less clear than those of ECM fungi (Sieber & Grünig, 2013). The high abundance in two-year moth feeding treatment may suggest opportunistic roles of *Meliniomyces* and *Phialocephala* as primary root colonizers shortly after damage (Heinonsalo, Buée, & Vaario, 2017; Sieber & Grünig, 2013). However, *Meliniomyces* and *Phialocephala* were also present in pot-grown seedling roots, suggesting that they may originate from the growth substrate of the seedlings. Their higher abundance in the roots of transplanted seedlings in the two-year moth feeding treatment could indicate lower colonization with other RAF originating from soil and roots of defoliated adult mountain birch compared to the longer (3-yr) moth feeding treatment level and the control. Higher mycorrhizal abundance in the three-year moth feeding treatment and control might also inhibit abundance of *Phialocephala* (Reininger & Sieber, 2012).

The higher abundance of ECM fungi in seedling roots in the three-year moth feeding treatment is hard to explain (Fig. 3 and Supplement S4 in II). According to previous studies, moth herbivory changes RAF community (Saravesi et al., 2015) and growth of fungal ECM mycelium (Parker et al., 2017). Also fertilization is known to reduce growth and change community composition of ECM fungi (Kjøller et al., 2012; Nilsson & Wallander, 2003). However, these impacts are not always so evident. Nicolás et al. (2017) found the proportion of ECM OTUs to decrease after nitrogen fertilization, but no changes were found in overall fungal or ECM communities. There are also studies, where no changes in ECM fungal community have been found after nitrogen fertilization (e.g. Hay, Phillips, Nicholson, & Jones, 2015). The results from the moth feeding experiment might be affected by the study set up, where the focus was on RAF communities in establishing seedlings. It might be that ECM fungi actively search for new carbon sources in the roots of transplanted seedlings when carbon allocation from the defoliated host tree decreases. In the two-year treatment, fungal mycelium had one year less time to react to changes in C allocation of the host (Fig. 2). The difference between the impacts of two-year and three-year moth feeding may also be due to a combination of moth feeding and variation in annual weather conditions. Previous studies have reported shifts in fungal communities under experimental warming (Clemmensen, Michelsen, Jonasson, & Shaver, 2006; Deslippe, Hartmann, Mohn, & Simard, 2011; Deslippe, Hartmann, Simard, & Mohn, 2012; Treseder et al., 2016), and especially ECM fungi have been reported to become more abundant (Clemmensen et al., 2006; Deslippe et al., 2011). At the time of the natural moth feeding experiment, the growing seasons 2011 and 2013 were exceptionally warm (Finnish Meteorological Institute, 2015; Table 2 in II). Possibly, the warm summer in 2011 could have increased the impact of the moth feeding in three-year moth feeding treatment, causing the abandonment of the previous host (the adult mountain birch) and the effective colonization of seedling roots with ECM, shortly after planting in 2012. The results considering the longer moth feeding treatment (3-yr), differ from the results of Karst et al. (2015), who found Helotiales (including *Phialocephala* and *Meliniomyces*) to be more common in the ECM community of *Pinus contorta* seedlings, when seedlings were grown in soil originating from beetle-killed sites compared to seedlings grown in soil originating from undisturbed sites. In the natural moth feeding experiment, this kind of change in RAF community was observed in the two-year moth feeding treatment, where no moth larvae had been before planting the seedlings. Natural moth feeding had only slight impacts on OTU numbers of some fungal functional guilds in soil (Hypothesis 2c; Fig. 3, Table 3 and Supplement S4 in II). Changes in RAF and soil fungal communities have been observed after insect outbreaks, and the changes to relate to decreases in carbon and increases in nutrient availability (Parker et al., 2017; Pec et al., 2017; Saravesi et al., 2015). Less studies concentrating on first inoculation of the seedling roots after insect outbreaks are available (e.g. Karst et al., 2015), but it is known that e.g. at clear-cuts availability of ECM symbionts reduces as the number of adult host trees decreases (Jones, Durall, & Cairney, 2003).

In the parallel simulated moth herbivory experiment in the *Betula* study (II), changes were observed in the composition and diversity of soil fungal community as a response to moth frass addition (F) (Hypothesis 2c; Fig. 4, Table 4 and

Supplement S8 in II). However, the responding fungal guild in soil was ERM fungi, that colonize roots of ericoid dwarf shrubs (Grelet et al., 2016). Organic soil at the study sites was formed by the dead roots and other parts of understory species, largely by parts of mountain crowberry (*Empetrum nigrum* ssp. *hermaphroditum)*. The most evident change in soil fungal community was found in the abundance of *Pezoloma* (formerly known as *Rhizoscyphus* / *Hymenoscyphus ericae*), which is known to be a common root symbiont of *Empetrum* (Grelet et al., 2016; Smith & Read, 2008). Abundance of *Pezoloma* increased due to moth frass addition, when its host plants (understory ericoid shrubs) were not damaged. No increase in the abundance of *Pezoloma* was detected, if moth frass addition was combined with defoliation and damaging treatment (DF). In addition, compensatory growth of *Empetrum* was observed in DF plots (Heiskanen et al., unpubl), suggesting higher carbon allocation to shoot and leaves instead of roots and root symbionts, affecting possibly the abundance of *Pezoloma*. In previous studies, multiple different impacts of nutrient amendments on *Pezoloma* have been reported. Morrison et al. (2016) found long-term nitrogen addition to increase abundance of *Pezoloma*. However, Johansson (2000) did not find fertilization to have impacts on ericoid fungal colonization of *Calluna.* Neither did Ishida and Nordin (2010) find changes in fungal communities of *Vaccinium myrtillus* and *V. vitis-idae* after nitrogen addition. Nitrogen enrichments have also been reported to decrease ericoid fungal colonization in *Calluna* roots (Yesmin, Gammack, & Cresser, 1996) and OTUs in *Geum* roots (Dean et al., 2014). These multiple different results may imply that the ability to use different kinds of nitrogen sources may differ between *Pezoloma* genotypes (Cairney, Sawyer, Sharples, & Meharg, 2000). Some genotypes of *Pezoloma* are known to be able to use fungal necromass as nitrogen source (Kerley & Read, 1998). However, *Pezoloma* is also known to increase plant growth after inorganic nitrogen amendments (Scagel, 2005). In nitrogen limited ecosystem, where moth outbreaks occur cyclically, mix of fungal partners that can utilize both organic nitrogen sources and inorganic nitrogen pulses would be most favourable to the host in a long term. Increase in *Pezoloma* likely imply presence of such fungal genotypes that can react fast to moth frass addition. Moth frass addition (F) also decreased abundance of saprotroph-pathogen fungi. Decrease in the saprotroph-pathogen guild may indicate that in this infertile system, fungal saprotrophs typically degrade recalcitrant litter (Lindahl & Tunlid, 2015), and they do not benefit from moth frass. As nutrients are more easily available, saprotrophs may proportionally decrease as other groups increase. Contrary to Hypothesis 2b, only a few impacts on RAF of mountain birch seedlings were found in the simulated moth herbivory experiment (Table 4 and Supplement S8 in II). Again, the results might be affected by the experimental set up, as discussed above in the case of moth feeding experiment. In general, results from the *Betula* study indicate close but complex interactions between plants and fungi and the soil environment (Bardgett & Wardle, 2010; Teste & Dickie, 2016), and that impacts of simulated and natural herbivory on soil fungi and RAF communities of establishing seedlings might be different in an experimental setup.

In the *Picea* study (III), RAF communities of Norway spruce seedlings grown in uncut forest (F) and those of seedlings grown at clear-cut sites had different compositions (Fig. 1 and Table 4 in III). Contrary to Hypothesis 3, no significant differences in RAF community composition between energy wood harvesting treatments (treatments P, RP, SRP) at clear-cuts were observed. Clear-cutting is usually accompanied by soil modification of the sites, and thus both aboveground and belowground parts of ecosystem are heavily disturbed. These kinds of environments have similarities with primary successional sites, e.g. from the fungal perspective, more open sites free from competition and hosts which are not yet attached to RAF networks. At the clear-cut sites, root fungal communities of spruce seedlings were dominated by early successional ECM fungi (e.g. *Thelephora, Amphinema*). These symbionts could originate from tree nurseries, soil propagules or wind-dispersed spores (Jones et al., 2003; Teste, Simard, & Durall, 2009). Furthermore, in the patch-mound, roots of tree seedlings are isolated from the roots of other plants (Jones, Hagerman, & Gillespie, 2002). Even if dwarf shrubs at the clear-cuts would host, for instance, root fungal endophytes that could colonize tree roots (e.g. Grelet et al., 2016), it takes time and energy for fungal mycelium to reach tree seedling roots due to the physical sand barrier. It is possible that this isolation strengthens the competitive advantage (the priority effect; Johnson, 2015; Kennedy, 2010; Vannette & Fukami, 2014) of nursery fungi in seedling roots. In the forest, high-biomass ECM fungal species typical of older successional stages (e.g. *Cortinarius*) were present in seedling roots, indicating the role of mature trees as a carbon source for these fungi.

According to the results of the *Betula* and *Picea* studies (II, III), it seems that the formation of the seedling RAF community is context dependent. Similar results have been obtained also in other studies (e.g. Karst et al., 2015; Moeller, Dickie, Peltzer, & Fukami, 2015). Moeller et al. (2015) concluded that the formation of ectomycorrhizal partnerships of the seedlings appeared to be facilitated by common mycorrhizal networks in the forest, whereas at the sites (grasslands), where the networks were absent, associations seemed to be formed primarily with spore-based colonists. In the *Picea* study, the abundance of *Thelephora*, which is a spore-based colonist, was high at the clear-cut sites. It appears that the symbionts, which are present first, are kept if they are beneficial and until outcompeted by other fungi. This has probably been the case in *Betula* and *Picea* studies, assuming that *Meliniomyces* and *Phialocephala* originated from the growth substrate and *Thelephora* from the tree nursery.

RAF diversity

Diversity of root and soil fungi is high. It has been estimated that only c. 10% of fungal species have been described (Bass & Richards, 2011; Blackwell, 2011; Hibbett et al., 2011). Fungal diversity studies have become easier as new methods have been developed (e.g. NGS). With NGS and bioinformatics, fungal DNA sequences representing communities in multiple samples can be grouped according to their similarity (e.g. 97%) to the molecular units. In AM fungal research, the term virtual taxa (Öpik et al., 2014) is best defined and established. In general, the term operational taxonomic unit (OTU, Shokralla et al., 2012; Tedersoo et al., 2014), is used in research and bioinformatics related to NGS methods. Through both concepts, unknown fungal diversity can be classified and patterns in fungal community dynamics detected. In the *Betula* and *Picea* studies, the impacts of disturbance on RAF diversity were studied using rarefied OTU richness and OTU based diversity indices.

In both *Betula* and *Picea* studies (II, III) disturbance had clear short-term effects on the diversity and the dominance of fungal guilds and taxonomic groups. Changes in richness, diversity and dominance in fungal OTU level seemed to relate to broader changes detected at the more general level of grouping in taxonomic groups and functional guilds. Some fungal groups increased their abundance and dominance in the community after disturbance. In the *Betula* study (II), abundance of Basidiomycetes and ECM fungi and total fungal dominance were higher in three-year moth feeding treatment, whereas in the two-year moth feeding treatment, abundance and OTU richness of Ascomycetes and total fungal OTU richness were higher and dominance lower (Supplement S4 in II). In the simulated experiment in the *Betula* study, mimicked moth defoliation and moth frass addition altered soil fungal diversity, instead of that in roots. Moth frass addition increased dominance of ERM fungi in soil and decreased total fungal diversity. Most notable changes were observed in the OTUs representing *Pezoloma* (Supplement S8 in II). The impact of moth frass addition (F) on ERM fungi was diminished by mimicked defoliation and shoot-damaging (DF). The results considering the soil fungal communities indicate the dominance of ERM fungi in subarctic forest-heath soil, and their responses to moth frass addition. In the *Picea* study (III), differences in RAF diversity in Norway spruce seedling roots between clear-cuts and uncut forest sites associated especially with changes in ECM fungi in the community (Table 2 in III). *Thelephora* dominated RAF communities of seedlings at clear-cuts, whereas in the forest total fungal OTU diversity and ECM richness in the seedling roots were higher (Table 4 in III).

Fertilization has been found to reduce richness in primary producer communities with low evenness in terrestrial systems (Hillebrand et al., 2007, and references therein), and to decrease diversity and to alter community composition of soil fungi (Allison, Hanson, & Treseder, 2007; Leff et al., 2015). Also in the *Betula* and *Picea* studies, disturbance had increasing impacts on total fungal OTU dominance (II: 3-yr moth feeding, moth frass addition; III: clear-cutting) and abundances of different fungal groups and genera within the communities (II: 3-yr moth feeding: ECM, Basidiomycota; moth frass addition: ERM, *Pezeloma*; III: clear-cutting: *Thelephora*). With new research methods, the data on fungal diversity and its relation to disturbance has started to accumulate (e.g. Glassman, Levine, DiRocco, Battles, & Bruns, 2016; Lekberg et al., 2012; Saravesi et al., 2015; Sun et al., 2015; Tolkkinen et al., 2015). However, the results considering disturbance-diversity-dominance relationships have been mixed: in some studies no connection between disturbance and diversity has been found (Lekberg et al., 2012), while in others fungal OTU diversity has decreased and dominance increased (Glassman et al., 2016; Tolkkinen et al., 2015) or diversity has been the highest shortly after disturbance (Sun et al., 2015).

Molecular methods have opened new possibilities to study biodiversity, and without them we would know only little about, e.g. soil microbial diversity. However, there are some small, but possibly relevant, points that should be acknowledged when diversity is measured based on OTUs. Firstly, we should understand, that as a concept, operational taxonomic unit at the level of 97% sequence similarity differs from the concept of the species. Genetic variation in the ITS region of RAF differs between the species or species groups, and no single threshold value works well in line with traditional morphology-based species criteria for all fungi (Nilsson, Kristiansson, Ryberg, Hallenberg, & Larsson, 2008). For example, a similarity of 97% is not high enough for identification of most *Cortinarius* species, and not all *Cortinarius* species can be detected with ITS1 or ITS2 regions (Garnica et al., 2016). Secondly, we should acknowledge that OTU clustering methods applied in the study may have impacts on the number of OTUs formed (Nguyen, Smith, Peay, & Kennedy, 2015). Consequently, changes in fungal OTU dominance and diversity may indicate in some cases, instead of true changes in species diversity, the presence of different taxonomic groups in the sample or the impacts of the OTU clustering method. Interpretation of OTU-based diversity results and formulating a comprehensive view on fungal diversity may be easier, if multiple research methods are applied at the same time to study fungal community (Powell & Sikes, 2014).

Impacts of disturbance intensity on RAF

The intermediate disturbance hypothesis (IDH, Connell, 1978; Grime, 1973) is one of the most well-known theories related to the disturbance-diversity relationship. According to IDH, diversity of competing species should be expected to be maximized under intermediate frequencies or intensities of disturbance or environmental change (see for a review, Shea et al., 2004). However, it has been questioned whether the IDH really takes place in nature or empirical studies (Fox, 2013; Hughes, Byrnes, Kimbro, & Stachowicz, 2007; Mackey & Currie, 2001). In the studies of this thesis, both AM and DSE fungal colonization types were present at the intermediate stages of succession in the *Deschampsia* study (Fig. 1 in I), and OTU diversity was the highest at the intermediate frequency of natural moth feeding (two-year moth feeding) in the *Betula* study (Supplement S4 in II). From the perspective of IDH, clear-cutting is at the intensive end of the scale and in theory it should, and in practice it did, decrease OTU diversity in spruce seedling roots in the *Picea* study (III). However, no differences were observed between the intensity levels of energy-wood harvesting practices in the short term. As an intensive disturbance, clear-cutting might have masked differences between intensity levels or a longer time would be required for their detection.

3.2 Interactions between soil environment, vegetation and RAF

Changes in RAF co-occurred with changes in the soil environment (I, III) and aboveground vegetation (I, II, III) (Question 2). In the *Deschampsia* (I) and *Picea* (III) studies, responses in RAF colonization or community composition correlated with changes in the soil environment and vegetation. In the *Betula* (II) study, no changes in soil nutrients were observed, but changes were found in RAF and soil fungal community composition (see the previous section 3.1).

Soil environment

In the *Deschampsia* study (I), soil-soluble P increased and pH decreased in parallel with a shift from AM fungal-dominated root colonization to DSE-dominated along a successional gradient (Supplement 1 in I). In addition to soil characteristics, the fungal shift is in line with the benefits that the host may gain from these fungi. At young successional stages, AM fungi are likely beneficial to wavy-hair grass growing in bare sand, as AM fungi effectively catch P with their extraradical hyphae (Smith & Read, 2008). DSE fungi are reported to be more beneficial for host plant growth when the organic forms of nutrients are dominant in soil (Mandyam & Jumpponen, 2005; Newsham, 2011). DSE fungi are known to be able to produce hydrolytic enzymes, which enable the usage of organic matter in their nutrition (Caldwell, Jumpponen, & Trappe, 2000; Mayerhofer, Fraser, & Kernaghan, 2015). DSE-type root endophyte inocula have also been shown to improve N uptake of wavy-hair grass (Zijlstra et al., 2005). According to present knowledge, AM fungi have a rather limited ability to release N from organic matter (although they can increase the transfer of mineralized inorganic N to plants, e.g., Hodge & Storer, 2015; Smith & Smith, 2011), which may also contribute to the observed pattern. As was found in the *Deschampsia* study, also Postma, Olsson, and Falkengren-Grerup (2007), and Francini, Männistö, Alaoja, and Kytöviita (2014) found decreases in AM fungal colonization with decreasing soil pH from young to older stages of succession.

In the *Betula* study (II), no impacts of moth frass addition or real moths were observed in soil nutrient levels (Supplement S1 section 1.1.2. in II), but frass addition in the simulated moth herbivory experiment increased abundance of ERM fungi in soil implying that nutrient pulse is rapidly incorporated in fungal biomass (see section 3.1; Fig. 4 and Supplement S8 in II). In natural moth herbivory gradients, outbreaks take place on wider spatial scales and therefore, clear changes also in soil nutrient levels are observed (Kaukonen et al., 2013). It might be that fertilizing impacts of experimental treatments in the *Betula* study did not correspond to those of real moth outbreaks due to the small scale of the experiment. It is possible that in natural gradients these impacts may accumulate across outbreak sites, thus being more intensive.

In the *Picea* study (III), RAF communities in the roots of Norway spruce seedlings were rather similar in all treatments at clear-cut sites (regardless of the type of energy wood harvesting) where the soil nutrient levels were high (Figures 1-3, Tables 2-4 in III). These communities clearly differed from the RAF communities of the seedlings grown in nutrient-poor forest soil. It is well known, that site preparation after clear-cutting may induce a significant release of soluble N from soil, and this effect may persist for decades after forest regeneration (Kubin, 1998, 2012). High soluble nutrient levels at clear-cuts may have strengthened the colonization of early successional ECM fungi (the priority effect; Johnson, 2015; Kennedy, 2010; Vannette & Fukami, 2014).

Vegetation

In the *Deschampsia* study (I), parallel successional shifts were observed in surrounding vegetation and RAF community of wavy-hair grass. With increasing successional age, coverages of dwarf shrubs increase in the field layer and tree species in the canopy. DSE fungi, especially PAC species, are known to be able to grow in the roots of grasses, dwarf shrubs and trees, as well as without the host as saprotrophs in organic soil (Sieber & Grünig, 2013). The higher DSE hyphal colonization in wavy-hair grass roots at older successional stages correlated with the presence of other potential host species, but may also be due to higher organic matter in soil. Soil organic layer formation may be connected with the presence of dwarf shrubs species decomposing slowly (Clemmensen et al., 2015; Wardle et al., 1997). In the *Picea* study (III), comparable correlations between soil environment, vegetation, and RAF communities were found. RAF of spruce seedlings were different between clear-cut sites and uncut forests, where also characteristics of soil environment, vegetation and other site conditions differed.

In the *Betula* study (II), changes in RAF communities of transplanted mountain birch seedlings were observed only in the natural moth feeding experiment, where moth larvae virtually killed older mountain birches in experimental plots. In the simulated moth herbivory experiment, mimicking manual defoliation did not have such a destructive effect on adult mountain birches. Compensatory growth of *Empetrum* was observed in the plots where established mountain birch was manually defoliated and understory vegetation mechanically damaged and moth frass added (DF). However, the increase in abundance of ERM fungi in soil was observed instead in plots, where only moth frass was added (F). The results of the *Betula* study show, that performance of the host plant has impacts on RAF communities, but that these impacts are context dependent. Although some changes in the vegetation of the experimental plots were observed after treatments in both experiments, these changes were not as severe as those after natural moth outbreak (see studies Kaukonen et al., 2013; Saravesi et al., 2015). This might be one explanation for the mixed results of the *Betula* study.

3.3 Spatial and temporal scales of plant-RAF interactions

In general, the results of this thesis draw attention to the context-dependency of RAF communities. Different successional drivers (e.g. also observed in *Deschampsia* study, I) occur in different spatial and temporal scales and these drivers are coupled (Meiners, Cadotte, et al., 2015; Teste & Dickie, 2016). For example, changes in fungal competition and plant host carbon allocation occur in hours, days and weeks whereas changes in soil conditions (pH, moisture, nutrient levels) and plant community may take years, decades and centuries (Teste & Dickie, 2016). The processes that RAF are involved (Fig. 4, Teste & Dickie, 2016), and the patterns, they display (e.g. Davison et al., 2016; Zobel, 1997) can be sorted out in different spatial and temporal scales. For example, different fungal guilds have important roles in ecosystem processes, such as carbon and nutrient cycling and soil formation (e.g. Clemmensen et al., 2015; Fernandez & Kennedy, 2016; van der Wal et al., 2013). On a community scale, RAF may increase the number and the sizes of realized niches in the plant community (Bever et al., 2010; Peay, 2016), alter interactions between organisms (Bennett et al., 2017; Dickie et al., 2017), connect root systems by forming symbioses and networks with more than one host (Klein et al., 2016), and affect plant coexistence (Jiang, Moore, Priyadarshi, & Classen, 2017). At the scale of individuals, RAF species harvest nutrients for the hosts and are rewarded with host-assimilated carbon, depending on environmental conditions (Smith & Read, 2008).

From the perspective of an individual seedling, fungal partners that are suitable for the current habitat are important. The first years of a seedling's life are crucial for its long-term establishment. Depending on the place of germination (e.g. nursery vs. natural site), the first fungal colonists might differ. In theory, if the partners are good enough, they should be rewarded and kept (Bever et al., 2010; Kiers et al., 2011). However, also fungal communities are affected by the environment. It might be that if soil nutrient conditions are not optimal, for e.g. nursery ECM fungi, they are outcompeted by ECM fungi associated with mature trees (e.g. in the *Picea* study (III) in uncut forest). The access to the wider RAF networks is likely beneficial to the seedling. For example, higher ectomycorrhizal colonization, received from established *Quercus* trees nearby, has been found to correlate with higher nutrient status and the growth of *Quercus* seedlings (Dickie

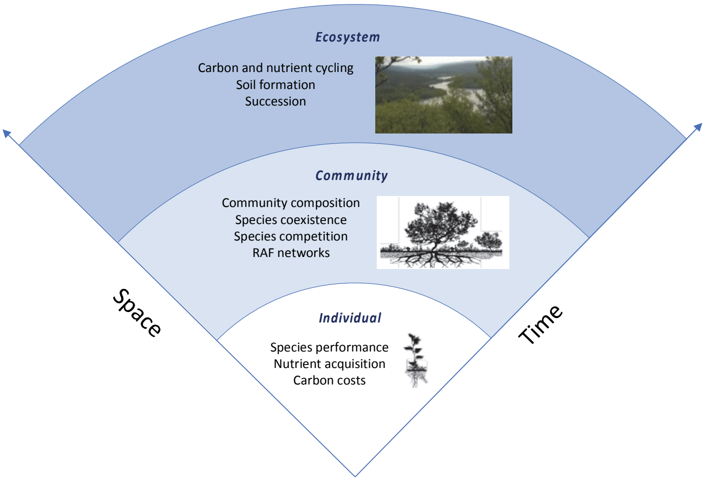


Fig. 4. Examples of the impacts of RAF on plant hosts on different ecological scales and their relation to time and space.

et al., 2002). Regardless of indirect facilitation, seedlings also compete for light, water and nutrients with mature trees, intensity of competition depending on distance between the seedling and mature tree (Bever et al., 2010; Dickie et al., 2002). Plant hosts associate with multiple and partly same fungal partners, and thus RAF networks may smoothen direct negative and positive interactions between host plants (Bever et al., 2010). It might be that rather than the interactions between the individual partners, the interactions between different parts of the networks are more important over time (Bascompte, Jordano, Melián, & Olesen, 2003). RAF partners and networks are known to change along succession (Barker, Simard, Jones, & Durall, 2013; Kyaschenko, Clemmensen, Hagenbo, Karltun, & Lindahl, 2017; LeDuc, Lilleskov, Horton, & Rothstein, 2013; Smith & Read, 2008; Sun et al., 2015; Twieg, Durall, & Simard, 2007; Varenius, Kårén, Lindahl, & Dahlberg, 2016). If the seedling is able to establish, survive the first years and form relatively good mutualistic associations with fungi, it may also reproduce one day.

RAF and their interactions with plants and other organisms have essential roles also at ecosystem level (e.g. Clemmensen et al., 2015; van der Wal et al., 2013). Impacts of RAF on ecosystem functions are both direct (e.g. carbon to fungal mycelia and soil nutrient levels) and indirect (e.g. growth and carbon assimilation of the host) and they take a place in ecosystem functioning through different ecological scales (Fig. 4). It is challenging to evaluate the importance of plant-RAF interactions from a wider perspective. RAF communities operate on very small spatial and time scales with their short life-cycles, whereas, for example, their host trees may live decades and centuries (Classen et al., 2015). Furthermore, cumulative and trophic-level-mediated impacts can be observed only over time. For example, the site history has impacts on characteristics of the current habitat, but also current species, species interactions and networks form partially the future history and habitats (Meiners, Cadotte, et al., 2015; Oliver et al., 2015). Indirect impacts of fungi mediated by their host plant might be good to be scaled to wider levels thorough impacts of the host (Classen et al., 2015).

The studies of this thesis concerned community level processes. The temporal scale of conducted studies (I, II, III) was 2–4 years, which is a short time at the scale of forest succession, but sufficient in detecting responses in species performance and interactions (e.g. in the *Betula* (II) and *Picea* (III) studies). According to the results, RAF communities seem to be affected by their habitat, abiotic conditions and biotic interactions and by disturbance that alters all the previous. This finding is in accordance with the species pool framework, according to which present species pool and species richness are determined by dispersal, abiotic and biotic filters (Zobel, 1997). Disturbances may shift communities from one state to another rapidly (Saravesi et al., 2015; Treu et al., 2014; White & Jentsch, 2001). In the studies of the thesis, RAF communities changed after disturbance or along succession. Increases in more opportunistic and early successional spore-base colonists were observed after disturbance being in line with previous studies (Karst et al., 2015; Teste, Simard, & Durall, 2009; Twieg et al., 2007). These observed changes in RAF may diminish over time (e.g. Dickie et al., 2013; Varenius et al., 2016), but in some cases ecosystems do not recover after intensive disturbance (Ammunét, Bylund, & Jepsen, 2015; Lehtonen & Heikkinen, 1995). Instead, they'll stay in a new, alternative stable state (van der Wal, Bardgett, Harrison, & Stien, 2004). Long-term studies are needed to understand the impacts of intensive disturbances (e.g. moth outbreaks) on wider spatial and temporal scales.

4 Conclusions

New study methods, such as NGS, have enabled more-specific quantification of diversity and dynamics of soil and root fungi. The results of this thesis highlight ecological roles of four RAF genera that increase or maintain a high abundance shortly after disturbance. These fungal genera were *Phialocephala*, *Meliniomyces*, *Thelephora* and *Pezoloma*. *Phialocephala* belong to the *Phialocephala-Acephala* species complex (Grünig et al., 2008). These species live as root endophytes (DSE) of multiple plant host species (grasses, dwarf shrubs, trees) or as saprotrophs in soil. *Meliniomyces* are known to be able to colonize roots of dwarf shrubs and trees, but to differ in their enzymatic profile from ECM fungal species (Grelet et al., 2016; Heinonsalo et al., 2017). Both *Phialocephala* and *Meliniomyces* colonized roots of vigorous mountain birch seedlings in the plots that were exposed to the moth larvae for the first time in the year of planting. Regardless of their origins (the substrate or surrounding forest soil), these fungi were not outcompeted by ECM fungi in the roots but they were more abundant in two-year treatment compared to the control. This suggests more opportunistic ecological roles of these root colonizers compared to ECM fungi (Karst et al., 2015). *Pezoloma* increased in abundance and dominance in mountain birch forest soil after moth frass addition. *Pezoloma ericae* forms ERM with ericoid dwarf shrubs. The result hints that *Pezoloma* may have a role in nutrient cycling of mountain birch forest heaths, possibly being linked with the dominance of *Empetrum* and other ericoid dwarf shrubs in the field layer. The ecological role of *Thelephora* as an early colonizer at the clear-cuts (Jones et al., 2003) was confirmed in the *Picea* study. Furthermore, the patterns considering presence of high-biomass ECM-fungi (e.g. *Cortinarius*) in uncut forest in the *Picea* study, confirm those observed in previous studies. These fungi colonized roots of Norway spruce seedlings in uncut forest. In the *Betula* study, abundances of ECM fungal species were more patchy, and confusingly their abundance was not the highest in the control but in the longer moth feeding treatment. The latter result indicates the context-dependency that is known to be characteristic of the formation of RAF communities of seedlings (e.g. Moeller et al., 2015).

Altogether, the results of the present study indicate connections between both abiotic and biotic environments and RAF, and host species viability and RAF. Abiotic environment is modified by biotic organisms along succession, but also local species pool is restricted by both abiotic and biotic filters (Zobel, 1997). The results of the *Deschampsia* study hint that rather than individual plant-plant interactions, combined successional changes in aboveground (e.g. plant functional groups, canopy closure) and belowground (e.g. organic soil formation, pH) environments direct plant-fungal interactions. Also in the *Picea* study, RAF communities reflected surrounding soil environment and vegetation. These results support the results of previous studies (e.g. Clemmensen et al., 2015). Although, on the wider scale, habitat and species availability restrict the species pool, at the organism level, carbon and nutrient availability seem to govern the dynamics of symbiotic relationships (Teste & Dickie, 2016). Fungi are heterotrophs, and carbon availability from the host trees or other host plants is vital for the formation and functioning of plant-fungal interactions. Vice versa, the plant host is, at least somewhat dependent on its root symbionts in nutrient acquisition (Smith & Read, 2008). Both moth herbivory in the *Betula* study and clear-cutting in the *Picea* study reduce carbon flow from the established host plants to the fungal community in roots and soil. Further, RAF community composition of transplanted seedlings of both tree species was altered, in accordance with the prior knowledge on plant-RAF interactions.

In a broader context, RAF-plant interactions have pivotal roles in ecosystem functions. However, on wider scales their impacts and responses are not well-understood, due to the complexity of these interactive networks. In many cases, changes in RAF-plant interactions seem to be context dependent, and thus hard to predict. In future, climate change will affect also RAF and their host plants. Understanding these interactions more broadly and estimating their impacts on ecosystem functions and responses to climate change require a holistic view, which may be achieved through studies on multiple ecological scales and collaboration between researchers studying different trophic levels and parts of ecosystems.

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Original publications

This thesis is based on the following publications and the manuscript, which are referred throughout the text by their Roman numerals:

I Huusko, K., Ruotsalainen, A. L., & Markkola, A. M. (2017). A shift from arbuscular mycorrhizal to dark septate endophytic colonization in *Deschampsia flexuosa* roots occurs along primary successional gradient. *Mycorrhiza*, 27(2), 129–138. doi:10.1007/s00572-016-0736-x

II Huusko, K., Ruotsalainen, A. L., Wäli, P. R., Andersson, T., Koivuniemi, H., Saravesi, K., Suokas, M., Suominen, O., & Markkola, A. M. Controlled natural and simulated herbivory show contrasting effects on soil and on root fungal communities in subarctic mountain birch forest. *Manuscript*.

III Huusko, K., Tarvainen, O., Saravesi, K., Pennanen, T., Fritze, H., Kubin, E., & Markkola, A. (2015). Short-term impacts of energy wood harvesting on ectomycorrhizal fungal communities of Norway spruce saplings. *The ISME Journal*, 9(3), 581–591. doi:10.1038/ismej.2014.154

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