Functional versatility and diversity in the plant root mycobiome

Ella Thoen

Dissertation presented for the degree of

Philosophiae Doctor (PhD)

2019

Department of Biosciences

Faculty of Mathematics and Natural Sciences

University of Oslo

Series of dissertations submitted to the
Faculty of Mathematics and Natural Sciences, University of Oslo
No. 2090

ISSN 1501-7710

Cover: Hanne Baadsgaard Utigard.
Print production: Reprosentralen, University of Oslo.


oslo mycology group

ACKNOWLEDGEMENTS

The last four years have been an exciting journey for me, personally and scientifically, towards what has in the end become this thesis, and there are so many people that have helped me through and made these years very enjoyable!

First of all, I would like to thank my main supervisor, Håvard Kauserud, for your constant encouragements, excellent advice and scientific discussions. Your excellent and inspiring lectures got me interested in mycology already during my bachelor, and I would not be where I am today, if not for you. I would not have managed without my co-supervisors, Unni Vik and Inger Skrede, with their constant encouragements and pep-talks. Unni, thank you for being the best office-mate ever, for all our little chats, and for all the advice, not to mention trusting me with parts of your beautifully designed project! Inger, thank you for always being there for a chat and for cheering me up when needed, and for all you wonderful ideas and help with my experimental designs. Thanks to the CIME-network and especially Mike Koomey for the opportunity to do this PhD.

Christoffer Bugge Harder, thank you for the opportunity to be part of your project, and to be able to work with your favourite fungi – the *Mycena*s. I, too, have learned to appreciate these small, beautiful mushrooms, and their ecological importance!

I am very much grateful for the invitation and welcome to Uppsala, where I worked with Audrius Menkis, whom was essential in teaching me techniques in fluorescent microscopy and radioactive isotope-labelling. Your openness and friendly way of being made me feel very welcome during both my stays at SLU in Uppsala.

I would like to thank the Oslo Mycology Group (OMG!) for all the valuable Tuesday meetings, the journal clubs, and the always helpful scientific discussion, but perhaps, most importantly for the excellent and fun work environment that each and every one of you is contributing to: our great lunches and coffee breaks, our laughs, our OMG dinner parties and the overall team spirit. You are all wonderful, and without you my years here would have been much less enjoyable! Many thanks to Marit, Synnøve, Solveig, Anders A, Anders K, Ida Marie, Dabao, Eva-Lena, Jørgen, Cecilie, Sandy, Jacky, Luis, Sunil, Jørn-Henrik, Marie, Perdro and all the rest of EVOGENE – you have all contributed to making this a great place to work. Especially, thanks to my wonderful master students, to whom I have had the honour of being co-supervisor: Simen, Lea, Åshild and Eivind. A great thank you to my so important friends from the bachelor-days and onwards: Runa, Ane, Torbjørn, Helga and the all the rest of Biobanden.

A big part of my PhD work has been teaching, and especially mycology in field courses. I would like to thank all the interested and enthusiastic students I have met during these years. Teaching duties has not really felt like a duty because of you. In the same context, I would especially like to thank Trond and Klaus for being excellent teachers in good, old field mycology, so that I now feel a lot more confident in determining fungal species in the field.

I would also like to express my gratitude towards all the interesting and interested people whom I have met in conferences and meetings around the world during the last year of my PhD, for encouraging chats at my poster, questions and comments on my talks, or discussions over dinner and drinks.

Outside of academia, I would like to thank my family, Mamma, Pappa and Frode *et al.*, firstly, for getting med interested in biology in the first place, but also for your constant support during all kinds

of ups and downs during the PhD. And thank you to my superb friends, Linn, Nora and Marion, for making my life more fun and helping me forget about science every once in a while.

Last but not least, thank you Erlend, for you endless support and love – and patience – during the entire PhD. Thank you for believing in me. I could never have done this without you!

TABLE OF CONTENTS

List of Papers

Paper I.

Climate structures belowground fungal communities in semi-natural grasslands

Ella Thoen, Synnøve S. Botnen, Letcia Pérez-Izquierdo, Line Nybakken, Aud Helen Halbritter Rechsteiner, Einar Heegaard, Vigdis Vandvik, Karina. E. Clemmensen, Håvard Kauserud, Unni Vik

Intended for Nature Ecology & Evolution

Paper II.

Versatility in fungi: *in vitro* evidence of ecological transitions in the genus *Mycena*

Ella Thoen, Christoffer Bugge Harder, Håvard Kauserud, Synnøve Botnen, Unni Vik, Andy F. S. Taylor, Audrius Menkis, Inger Skrede

Under review in New Phytologist

Paper III.

A single ectomycorrhizal plant root system includes a diverse and spatially structured fungal community

Ella Thoen, Anders B. Aas, Unni Vik, Anne K. Brysting, Inger Skrede, Tor Carlsen, Håvard Kauserud

Under review in Mycorrhiza

SUMMARY

Belowground fungal communities are highly complex systems, comprising a wide variety of ecologically and taxonomically different fungi distributed across various spatiotemporal scales. Saprotrophic fungi, decomposing dead organic matter, and mycorrhizal fungi, acquiring freshly synthesized carbon through mutualistic relationships with plants, are dominant functional groups in soil. However, several recent studies indicate that the borders between these ecological groups are far more blurry than previously appreciated. Mycorrhizal species have retained a repertoire of genes related to decomposition, and saprotrophic fungi may occupy healthy plant roots. In this thesis, belowground fungal communities were investigated, with emphasis on root-associated fungal communities and their functional roles.

In Paper I, we assess fungal communities in plant roots and soil in semi-natural grasslands by high-throughput sequencing (HTS). Utilizing a steep gradient in temperature and precipitation in western Norway, we aimed at investigating how climate variation, and in a longer time perspective – climate change, affect belowground fungal communities, as future projections for this region predicts a warmer and wetter climate. Across the gradient, we sampled soil and plant roots from the plants *Bistorta vivipara* and *Potentilla erecta*, which differ in mycorrhizal type. We measured soil edaphic factors, fungal enzymatic activities and ergosterol, a proxy for fungal biomass. While root-associated fungal communities were structured by both temperature and precipitation, soil fungal communities were only significantly correlated with temperature. The strongest effect of climate was seen when soil and plant roots samples were analyzed together. Fungal biomass, as well as soil carbon content, increased with decreasing precipitation, which could largely be explained by higher relative abundances of root-associated ascomycetes and slower turnover of fungal biomass. The predicted warmer and wetter climate may thus lead to lower soil carbon stocks in semi-natural grasslands in this region.

Amongst the most widespread genera in the *P. erecta* roots was the assumed saprotrophic *Mycena*. There are also numerous other reports on saprotrophic fungi occupying healthy plant roots. In paper II, we investigated interactions between seedling roots of *Betula pendula*, and a selection of *Mycena* spp. Using microcosms experiments, we investigated the physical interaction between host plant roots and the fungal cultures using differential staining of plant and fungal material and fluorescent microscopy. In a second experiment, we used radioactive isotopes and radiographs to trace potential transfer of nutrients between plant roots and the associated fungus. We show that all investigated *Mycena* spp. are capable of occupying living plant roots, and that at least one of the species included, *Mycena pura*, was capable of transferring radioactive ^{32}P to the seedling. Our study supports the view that the genus is not purely saprotrophic, and several species may occupy a transitional state between saprotrophy and biotrophy.

Due to the hidden lifestyle of belowground fungi, very little is known about the structure of root-associated fungal communities on a very fine scale. In Paper III we investigated the fine-scale structure of the root-associated fungal community of the ectomycorrhizal (EcM) host plant *B. vivipara*. Because the rhizome of *B.* vivipara grows directionally belowground, it was possible to include an age aspect and to assess whether a successional pattern among the fungi can be seen. We assumed that root tips sampled in sequence (i.e. neighbors) on a root thread where closer to each other in the soil, than root tips sampled further apparat. We recovered 41 operational taxonomic units (OTUs) that showed strong spatial structure within the root system. More neighboring root tips sharing the same OTU, consistent with spatial clustering, was observed in the youngest part of the root system than in the older. This may suggest a succession or fragmentation of the root-associated fungi even at a very small scale, where competition may be related to successional stages within the root system.

Overall, the papers in this thesis report on diverse plant root-associated communities, with versatile functions. As predefined niches are being challenged, we should aim be open-minded about putative ecological functions of the plant root mycobiome.

INTRODUCTION

People are categorical and tend to label things and put them in to predefined 'boxes'. Although these 'boxes' are practical for keeping order in a complex world, they often are too restrictive for biological entities. As an example, people are often either defined as introvert or extrovert, but the 'in-betweens' are seldom discussed, although they do exist. There often is an assumption that you cannot occupy more than one 'box'. The same could be said to be true for the study of ecology, where we tend to group organisms into predefined ecological niches. For fungi, however, several recent studies, suggest that the picture is not so clear, and that the borders between these predefined 'boxes' are often much more blurry than we previously have assumed.

Although often considered as microbiology, fungal ecology is in the midpoint between macro- and microbiology. In fact, the largest organisms on earth, both in terms of biomass and size, is the fungal mycelium of an individual of the species *Armillaria gallica*, spanning more than 35 hectares, weighing at least 40,000 kg and being at least 2500 years old (Smith *et al.*, 1992; Anderson *et al.*, 2018). Although fungal individuals can be large, and thus represent macro-organisms, hyphae, the fungal cells, are only a few µm in diameter. Fungal hyphae interacts with other organism on this microscopic scale (Bogar & Peay, 2017), and in that sense, fungi can be defined as microorganisms.

Traditionally, fungal communities in nature were studied as macro-organisms. Sporocarps were collected in the field and determined to species based on morphology, similar to plant vegetation surveys. Due to the complex lifecycles of fungi, microscopic inconspicuous fungi or fungi with cryptic lifecycles without forming larger sporocarps were hard to detect. Thus, only visible mushrooms, fruiting at the particular time of sampling were investigated. From the early 1990's, sequencing of DNA made it possible to determine species based on the order of nucleotides (Sanger & Coulson, 1975). Individual species could be isolated from environmental samples, such as roots or soil, and determined based on DNA sequences. This unravelled cryptic and previously undiscovered species (Vilgalys & Sun, 1994; Taylor *et al.*, 2000) as well as novel ecological roles for already described fungal species (Vrålstad *et al.*, 1998). With the 'molecular revolution' in the early 1990's (Horton & Bruns, 2001), fungal ecology took a new direction, and relatively large datasets could be produced from environmental samples (O'Brien *et al.*, 2005). With intensive work effort by the researcher community on tedious and expensive sequencing, it became progressively clearer that the unseen and cryptic diversity of fungi was much larger than what had been previously observed from sporocarps surveys or cultivation techniques (O'Brien *et al.*, 2005; Blackwell, 2011; Nilsson *et al.*, 2018).

In the late 2000's, the fungal ecologists embraced high throughput sequencing (HTS) methods and metabarcoding (Nilsson *et al.*, 2018), where millions of DNA sequence reads can be produced from environmental samples in a single sequencing run (Reuter *et al.*, 2015). Metabarcoding refers to

sequencing one specific gene region (called the barcode, i.e. barcoding) on a mixed DNA template. These large dataset of DNA barcodes can be used to describe fungal ecological patterns in nature (Bahram *et al.*, 2011; Averill *et al.*, 2014; Botnen *et al.*, 2014; Tedersoo *et al.*, 2014; Kyaschenko *et al.*, 2017; Fernandez & Kennedy, 2018). Although we are beginning to get a more comprehensive picture of fungal communities in a range of environments, fungal ecology is still to a large extent explorative and descriptive. An increasing number of molecular studies are demonstrating that the taxonomic and functional diversity of belowground fungal communities are extremely varied (Toju *et al.*, 2013; Baldrian, 2016; Kyaschenko *et al.*, 2017; Kohout *et al.*, 2018), new fungal lineages are discovered and described (Schadt *et al.*, 2003; Rosling *et al.*, 2011) and previous strictly defined niches are being challenged (Lofgren *et al.*, 2018; Schneider-Maunoury *et al.*, 2018). There is, of course, challenges related to studying the largely hidden and unseen diversity and functions of fungal communities in soils and belowground, which I will discuss in this thesis.

Ecology of soil fungi

Soil fungi comprise a wide group of ecologically very different species, which have commonly been grouped into functional guilds or trophic mode according to their suggested mode of nutrient acquisition (Nguyen *et al.*, 2016). Among the most commonly reported ecological roles in soil, are symbiotic fungi that acquire freshly synthesized carbon from the roots of host plants (Smith & Read, 2008; van der Heijden *et al.*, 2015) and saprotrophic fungi that decompose dead organic matter to access carbon (Baldrian & Valášková, 2008).

Most plants associate with beneficial symbiotic fungi on root level, an interaction termed mycorrhiza. Mycorrhizal fungi increases plant growth and uptake of mineral nutrients, and in return the fungi receive sugars from the plants (Smith & Read, 2008; van der Heijden *et al.*, 2015). Mycorrhiza is crucial for survival of the associated plants in natural environments, and evolved alongside Plantae's colonization of land (Strullu-Derrien *et al.*, 2018). This form of mutualistic symbiosis is widespread, including phylogenetically diverse groups of fungi and more than 80% of all higher plant species (Brundrett & Tedersoo, 2018). Mycorrhizal fungi are important for long-term storage of carbon (C) in boreal forest systems (Clemmensen *et al.*, 2013; Soudzilovskaia *et al.*, 2018) but are also efficient recyclers of organic material and mineral nutrients (Op De Beeck *et al.*, 2018; Sterkenburg *et al.*, 2018). As key players in soil ecology, knowledge of mycorrhizal fungi should be integrated in our understanding of plant ecology (Dahlberg, 2001; Koide *et al.*, 2014).

The most common form of mycorrhiza, arbuscular mycorrhiza (AM), is formed by 72% of all plants (Brundrett & Tedersoo, 2018) and exclusively by Glomeromycota and Mucoromycota (Brundrett & Tedersoo, 2018). It is also the oldest type of mycorrhiza, and was probably crucial in Plantae's colonization of the terrestrial habitat (Wang *et al.*, 2010). Plantae's genetic basis for forming

mycorrhizas with fungi likely predates the first land plants (Delaux *et al.*, 2013), and the oldest fossil of Glomean fungal spores is over 460 million year old (Redecker & Blackwell, 2000). In AM, the fungal hyphae penetrate the plant cell wall and form characteristic structures for nutrient exchange, termed arbuscles.

In contrast to AM fungi, ectomycorrhizal (EcM) fungi never penetrates the plant cell wall, and nutrient exchange is thus restricted to across the cell walls of the partners. The EcM symbiosis is characterized by a hyphal sheet, mantle, encapsulating the plant root tip, and an intercellular network of hyphae between plant cells in the root cortex, termed Hartig net (Fig. 1), where the nutrient exchange takes place.

Although only 2% of all plants form EcM, most ecologically and economically important trees in boreal and temperate climatic zones forms this form this type of symbiosis with fungi (Brundrett & Tedersoo, 2018). EcM communities can be highly diverse, even at small scales. For instance, a single EcM forming tree may host up to 200 fungal species as well as several individuals within a species (Bahram *et al.*, 2011). The reasons for why EcM host plants have such a diverse root-associated community is yet unknown, but it has been suggested that a diverse EcM community may act as a buffer against changing environmental conditions (Pena *et al.*, 2010). Fungal species involved in this symbiosis comprise a wide number of phylogenetically diverse species within Ascomycota and Basidiomycota, and has evolved independently from a saprotrophic lifestyle at least 78 times (Smith & Read, 2008; Tedersoo *et al.*, 2010; Kohler *et al.*, 2015).

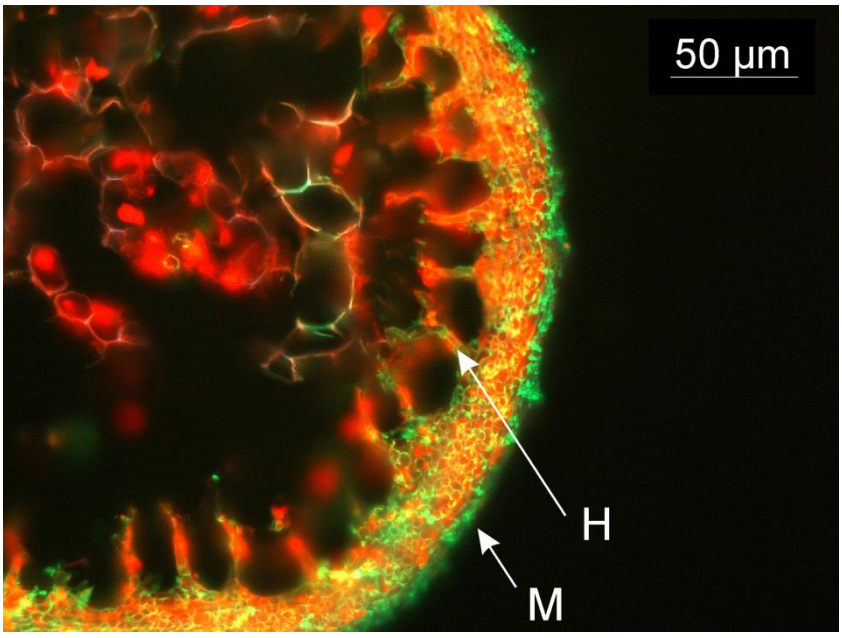

Figure 1 Cross section of an EcM root tip using fluorescent microscopy with differential staining of fungal (green) and plant (red) cells clearly show the thick hyphal mantel (M) and the inter-cellular hyphal network termed Hartig net (H). Photo: Ella Thoen

Not all plants are mycorrhizal, but all plants associate with fungi. All tissues of plants, including roots, are asymptotically occupied by fungi. These fungi do not have any common phylogenetic ancestor, but are polyphyletically grouped together on the basis that they live asymptotically inside plants – they are endophytes (Rodriguez *et al.*, 2009). Root-endophytes are

fungi that live inside plant roots without causing any structural changes to the root, without producing specific organs for nutrient exchange, and without being pathogenic (Brundrett, 2006). The function of root-endophytes may be very variable, but are often classified as either beneficial or commensalists, or their functions are unknown. Root-endophytes have been shown to maintain a repertoire of genes linked to both saprotrophic and pathogenic lifestyles (Schlegel *et al.*, 2016), and thus their functions may be context dependent. By the use of molecular studies, fungi that are supposedly saprotrophic, living on dead organic material, are be recovered from healthy plant root samples (Menkis *et al.*, 2005; Selosse *et al.*, 2009; Yao *et al.*, 2013; Liao *et al.*, 2014), and there is growing evidence that saprotrophic fungi may associate biotrophically with plant roots *in vitro* (Smith *et al.*, 2017). Mycorrhizal fungi have evolved from saprotrophic ancestors (Tedersoo *et al.*, 2010; Wolfe *et al.*, 2012; Kohler *et al.*, 2015), a process that likely is still ongoing, and several species may occupy a transitional state. Thus, our definitions of these functional guilds may be somewhat restrictive, and predispose us to prejudging the actual ecological functions of fungi recovered from different substrates.

Belowground structure of fungal communities

An increasing amount of studies are aiming at explaining what structures the fungal communities spatially in soil and plant roots on scales ranging from local (Lilleskov *et al.*, 2004; Blaalid *et al.*, 2012; Pickles *et al.*, 2012; Yoshida *et al.*, 2014), regional (Jarvis *et al.*, 2013; Miyamoto *et al.*, 2015) to global (Tedersoo *et al.*, 2014; Bahram *et al.*, 2018). On larger scales, climate and edaphic factors (such as pH) are among the important drivers in structuring fungal communities and phenology (Tedersoo *et al.*, 2014; Baldrian, 2016; Andrew *et al.*, 2018; Bahram *et al.*, 2018), and ecological processes that operate at scales from hundreds to thousands kilometres affect fungal community composition (Peay *et al.*, 2016). Because soil and root-associated fungi are important regulators of soil carbon dynamics (Clemmensen *et al.*, 2013; Averill & Hawkes, 2016; Hagenbo *et al.*, 2017), understanding the main drivers structuring fungal communities and community-related decomposition processes are important, especially in the light of climate change (Crowther *et al.*, 2016). On smaller scales, fungal communities are patchily distributed (Tedersoo *et al.*, 2003; Lilleskov *et al.*, 2004; Pickles *et al.*, 2012), and can be highly diverse even at a single host plant scale (Bahram *et al.*, 2011; Yoshida *et al.*, 2014). We know relatively little about what structures fungal communities on scales representing the individual hyphae or individual mycorrhizas, but competition and dispersal stochasticity are both likely important factors (Kennedy *et al.*, 2009; Pickles *et al.*, 2012; Bogar & Peay, 2017). *Priority effects*, meaning that an early arriving species has an advantage in colonizing the habitat compared to later arriving species, has been deemed as a major factor in structuring fungal communities (Kennedy & Bruns, 2005; Yoshida *et al.*, 2014; Hiscox *et al.*, 2015). Understanding how fungi interact

belowground on these small scales, may be just as important for understanding large-scale pattern and belowground dynamics.

The overall aim of my thesis was to gain deeper knowledge about what structures belowground fungal communities on different spatial scales and investigate possible ecological functions of root-associated fungi. I have included both the descriptive molecular methodologies, as well as utilized experimental setups to test hypotheses about functions of root-associated fungi. By applying a broad selection of methods, I attempt to fill some of our knowledge-gaps in fungal ecology.

MATERIAL AND METHODS

There are several approaches to study soil- and root-associated fungal communities, and the choice of methods very much depends on the questions you are asking, which in turn will affect what you will be able to answer. Methods for studying soil fungi in natural settings differ substantially from *in vitro* experiments, but both approaches have their advantages and disadvantages. If you are interested in, for instance, EcM fungi, you would choose different methods than if you were interested in AM fungi, both in terms of host plant and molecular markers. In this section, I will outline the main methodologies used in the three papers in this thesis, the rationale behind, and their advantages and disadvantages. The focus will be mainly on root-associated fungal communities.

SOIL AND ROOT-ASSOCIATED FUNGAL COMMUNITIES FROM ENVIRONMENTAL SAMPLES

Since all plants associate with fungi in one way or another, and specific plant genera form different types of mycorrhizae (Brundrett & Tedersoo, 2018), choosing your host plant for investigating root-associated fungi is dependent on what questions you are asking. For instance, most plant species that

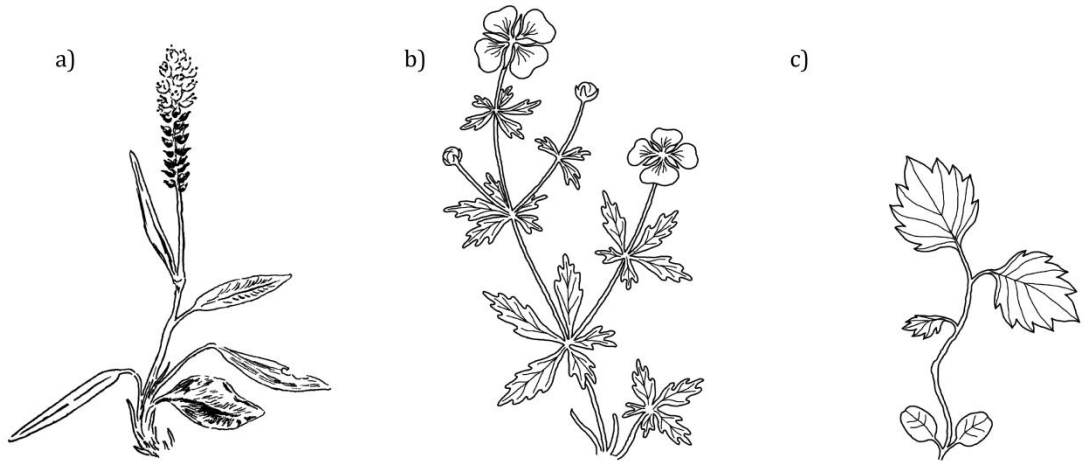

Figure 2 Schematic drawing of the three host plants used in this thesis. Two host plants were used to study root-associated communities, the ectomycorrhizal host plant *Bistorta vivipara* (a) in Paper I and III and the arbuscular- or non-mycorrhizal plant *Potentilla erecta* (b) in Paper I. Seedlings of *Betula pendula pen dula* (c) was used for an experimental setup in Paper II, looking at putative plant-fungus interactions at root level. Figure credit: Erlend Y. Fines

form EcM are trees and shrubs with very large root systems, and investigation of all root-associated fungi of adult EcM plants is therefore a tedious task. Several studies on EcM fungi have therefore focused on seedlings (Kennedy & Bruns, 2005; Menkis *et al.*, 2005; Yoshida *et al.*, 2014; Smith *et al.*, 2017), or on soil cores with roots collected around larger trees when investigating adult plants (Bahram *et al.*, 2011).

Although most plants forming EcM are trees and shrubs (Brundrett & Tedersoo, 2018) there are a few herbaceous plants that form EcM, among them the perennial ***Bistorta vivipara*** (L.) Delarbre. The small and condensed root system of this plant makes it ideal for studying the entire root system of adult EcM plants. Several studies have already confirmed the high diversity of root-associated fungi of this plant (Massicotte *et al.* 1998; Blaalid *et al.* 2012, 2014; Kauserud *et al.* 2012; Yao *et al.* 2013; Botnen *et al.* 2014; Mundra & Halvorsen 2015), comprising a wide range of both arbuscular- and ectomycorrhizal fungi as well as saprotrophs, parasites and endophytes.

In Paper I and Paper III we used *B. vivipara* (Fig 2a) to investigate root-associated fungal communities of EcM plants. *Bistorta vivipara* grows with a belowground rhizome, where growth is restricted to the proximal end (i.e. where the leaves and flowers sprout from). In Paper I, we also investigated the root-associated fungi of ***Potentilla erecta*** (L.) Raeusch. (Fig. 2b), which has been reported both as AM and as non-mycorrhizal (reviewed in Wang & Qiu, 2006), to be able to compare whether the same environmental drivers are structuring the fungal communities of plants with differing mycorrhizal type.

Root-associated fungal communities are also present in soil as mycelium, and soil samples as a substrate for studying root-associated fungi is therefore another possibility (e.g. Clemmensen & Michelsen, 2006; Sterkenburg *et al.*, 2018). In addition to capturing symbiotic root-associated fungi, all other trophic modes are captured as well (e.g. saprotrophic fungi and pathogens on soil fauna), and one could argue that you are closer to capture the entire fungal community as compared to looking at plant roots alone (although this is dependent on pre-processing such as sieving; see below). However, there are large discrepancies between the abundance of mycelium in soil and number of root tips occupied by EcM fungi (Kjøller, 2006), and looking at soil alone may give a different picture than if plant roots are included. Thus, it may be important to look at more than one sample type to gain a more complete picture of belowground processes, which we discuss in Paper I.

Typically, in fungal ecology, environmental samples are collected in the field and processed before molecular methods and downstream analyses (Lindahl *et al.*, 2013). As molecular work often is done on very small amounts of substrate (mg-g), careful homogenization of the sample is crucial. When studying fungal communities in plant roots, soil and debris attached to the roots must be removed, and when looking at individual plant species, roots must be disentangled from other plant roots. The root

systems studied in Paper I and Paper III were carefully rinsed, and all soil attached to the roots were removed under a dissection microscope, to avoid contaminations from soil fungi that are not living inside the roots. In Paper I, the entire root system of each individual plant was homogenized by bead beating, which has been shown to provide consistent results for replicate subsamples (Kauserud *et al.*, 2012). In Paper III we were interested in spatial structure of the root-associated fungal community within the root system. We therefore mapped and sampled root tips colonized by fungi from each root thread instead of homogenizing the entire root system.

Fungal communities are patchily distributed in soils (Lilleskov *et al.*, 2004; Pickles *et al.*, 2012), and typically several soil samples are taken at each plot and pooled in order to obtain a more representative sample (Clemmensen *et al.*, 2006; Voříšková *et al.*, 2014). Subsequently, the soil samples need to be homogenized, either by manual mixing or sieving and mixing, and a small subsample is then taken from the larger sample for DNA analyses. Sieving will remove larger fractions, such as stones, pebbles and roots from the soil, but this may also be manually removed when mixing without sieving. Thus, the processing of the soil will affect which parts of the fungal community you recover (i.e. inclusion of roots will include root-associated fungi to larger degree). DNA is then extracted from a subsample of the homogenized soil.

Molecular Methods

The study of fungal communities from environmental samples is often very much dependent on molecular methods. The fungal barcode for species identification is the ribosomal internal transcribed spacer (ITS) region (Schoch *et al.*, 2012), which has high intraspecific variation, but is relatively invariable within species. The ITS region consists of two highly variable regions, ITS1 and ITS2, which are flanked by conserved genes (18S and 28S), and separated by a conserved region (5.8S). This allows for primers that can either amplify the entire ITS region (White *et al.*, 1990; Gardes & Bruns, 1993), or ITS1 or ITS2 separately (Tedersoo & Lindahl, 2016). However, the primers used to amplify the regions have biases towards specific fungal groups, and fail to capture others (Begerow *et al.*, 2010; Tedersoo & Lindahl, 2016; Nilsson *et al.*, 2018). Additionally, some fungal taxa have very high intraspecific variation in the ITS region (Nilsson *et al.*, 2008), which may inflate operational taxonomic unit (OTU, a proxy for species) richness. Thus, the units you are investigating may not necessarily represent a real biological species. In HTS surveys, ITS1 or ITS2 are used as barcodes, although there is no consensus on which region should be preferred (Bellemain *et al.*, 2010; Blaalid *et al.*, 2013; Tedersoo & Lindahl, 2016).

Although HTS and metabarcoding now are established as routine operations, Sanger sequencing is still a valuable approach in some cases; Sanger sequencing can produce high-quality DNA reads from a single DNA template, either from fruit-bodies, pure cultures or from root-tips occupied by a single

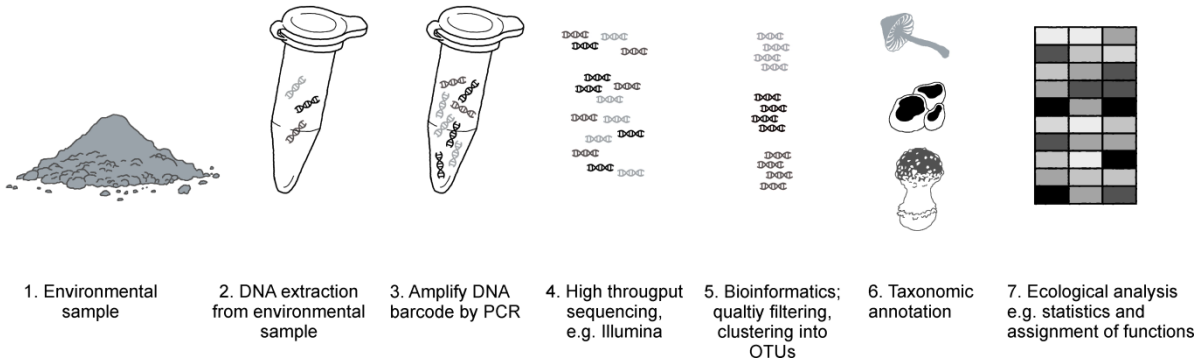

Figure 3. Illustration of the workflow using high throughput sequencing and metabarcoding. Figure modified for fungal studies from http://www.naturemetrics.co.uk. Figure credit: Erlend Y. Fines

(or dominating) species of fungi. The DNA sequence can then be identified by comparing it to sequences from known species, using for instance the fungal database UNITE (Kõljalg *et al.*, 2013). Sanger sequencing is limited to sequencing a single DNA fragment per run, and does not work with mixed samples (i.e. mixed template samples have to be cloned to isolate individual DNA fragments). Sanger sequencing can however, produce higher quality DNA reads of longer read length than most HTS platforms available (Reuter *et al.*, 2015).

Utilizing HTS methods and metabarcoding on environmental samples has now become the gold standard in microbial ecology, because you can amplify and sequence fungal DNA from mixed template sample without the tedious step of cloning individual DNA fragments, and the output greatly outnumbers what you can get from traditional Sanger sequencing. The concept is relatively straight forward (Fig. 3): DNA is extracted from an environmental sample, amplified using fungal-specific primers and sequenced on a HTS platform of your choice (Reuter *et al.*, 2015). Further, the sequence reads obtained are subjected to a number of bioinformatics analyses, including quality filtering, clustering of sequence reads into operational taxonomic units (OTUs) based on similarity and taxonomic annotation of the OTUs. There are numerous biases and problems at each step in the process, some of which are intrinsic to the method, and others that can be avoided by good scientific practice and careful selection of methods in each step (Nilsson *et al.*, 2018). Most of these issues have been discussed elsewhere (e. g. Nilsson *et al.*, 2008, 2016; Blaalid *et al.*, 2013; Lindahl *et al.*, 2013; Tedersoo & Lindahl, 2016; Bjørnsgaard Aas *et al.*, 2017; Botnen *et al.*, 2018), but I will touch upon aspects relating to the papers in the discussion.

THE EXPERIMENTAL APPROACH: *IN VITRO* INOCULATIONS

Although DNA based studies are powerful in describing fungal communities from environmental samples, studying fungal distribution and investigating drivers structuring the communities (Baldrian

et al., 2012; Lindahl *et al.*, 2013; Tedersoo *et al.*, 2014; Clemmensen *et al.*, 2015; Nilsson *et al.*, 2018), they tell us relatively little in terms of the actual functional roles that fungal species perform in nature. To test hypotheses about species ecology, experiments under controlled and simplified conditions are useful. Traditionally, *in vitro* re-synthesis experiments were used to confirm mycorrhizal status of fungi (Laiho, 1970), investigate growth benefits by mycorrhizal formation to plants (Daft & Nicolson, 1966; Laiho, 1970) and nutrient transfer between plant and fungus (Melin & Nilsson, 1950; Melin *et al.*, 1958). Nowadays, it's more commonly used for investigation of gene expression during symbiosis formation (Ceccaroli *et al.*, 2015; Kohler *et al.*, 2015; Martino *et al.*, 2018), but also for investigating putative biotrophic interactions between fungi and plants (Smith *et al.*, 2017). In *in vitro* experiments one can control for nutrient availability, light and moisture conditions that cannot be precisely controlled for in nature. Natural ecosystems are generally too complex to be able to control for all possible confounding factors. Additionally, you can control for which organisms you want include in *in vitro* experiments, which makes it possible to investigate specific interactions, without having to deal with unnecessary variability from other factors, biotic or abiotic, that may directly or indirectly influence the interactions.

In Paper II we used an experimental setup with seedlings of the EcM forming plant ***Betula pendula*** Roth (Fig. 2c), to investigate interactions between the seedlings and the assumed saprotrophic fungal genus *Mycena*. *Betula pendula* readily form EcM with a variety of different species of fungi (Le Quéré *et al.*, 2005; Tedersoo *et al.*, 2008; Kasurinen *et al.*, 2016). The generalistic properties of *B. pendula* make it ideal for investigating root-associations of fungi when exploring possible root-associations. Seedlings are practical in terms of size, and they can easily be kept sterile by germination from sterilized seeds.

THE PAPERS

The overall aim of this PhD thesis was to investigate the potential different functions of root-associated fungi, both across different spatial scales, ranging from cm in the soil (Paper III) to over 100 km (Paper I), and even across previously defined ecological groups (Paper II). A broad selection of methods were used for the different studies, ranging from classical Sanger sequencing of individual samples (Paper III) to HTS sequencing of soil and plant roots (Paper I), from natural environments (Paper I & III) to axenic laboratory experiments (Paper II).

In the first paper, we took advantage of a steep gradient in temperature and precipitation, to investigate how fungal communities in soil and plant roots of *P. erecta* and *B. vivipara* change across a climatic gradient in western Norway. The aim was to investigate whether climatic factors such as precipitation and temperature affect the fungal community in terms of community composition and functions (i.e. trophic modes), and what potential consequences this can have in a changing climate.

Figure 3. Often found fruiting among decaying litter on the forest floor, the *Mycena* spp. have by default been assigned to the saprotrophic guild. However, *Mycena* spp. may play potentially important roles within plant roots, where they are often found in HTS-studies. Photo: Ella Thoen

In the second paper, the setting was moved from natural environments to a controlled, axenic laboratory setting, to investigate further why a putatively saprotrophic genus of fungi is often found in healthy plant roots, using fluorescent microscopy of fine-roots and radioactive isotopes for tracing transfer of nutrients.

In the third paper, we looked at what structures fungal communities on a very fine scale. Using Sanger sequencing to identify EcM root tips of a single root system of *B. vivipara*, we investigated how these root-associated fungi were spatially structured at less than cm scales within a root system.

In the following section, I will briefly summarize the aims and results from the three papers included in this thesis.

PAPER I: CLIMATE STRUCTURES BELOWGROUND FUNGAL COMMUNITIES IN SEMI-NATURAL GRASSLANDS

Climate change is an imminent threat to biodiversity and ecosystem functioning globally. Soil fungi are key players in terrestrial ecosystems and several studies point to their importance for long-term sequestration of carbon (C) in boreal soils and soil C dynamics. We have limited knowledge about how climate change will affect fungal communities in boreal grasslands. In this study, we aimed to investigate whether fungal community composition in soil and plant roots are affected by climate. Several studies have looked at how climate change may alter fungal community composition in boreal forests and alpine tundra, but relatively few studies have investigated the impacts of climate on grasslands in boreal systems. We took advantage of steep climatic gradients in western Norway to assess the effects of variation in summer temperature and annual precipitation regimes on fungal community composition, activity and biomass in semi-natural grasslands. We sampled soils and plant roots from *Bistorta vivipara* and *Potentilla erecta* in 12 sites across the climatic gradient. While communities of fungi closely associated with plant roots were structured by both temperature and precipitation, soil-dwelling fungi only respond to changes in temperature. We found higher fungal biomass and soil C in drier climates, which could largely be explained by higher relative abundances of melanized root endophytes, climate-induced fungal enzymatic activity and slower turnover of fungal biomass. This may have consequences for the global C dynamics, as the predicted warmer and wetter climate in boreal areas may lead to lower soil C stocks in boreal grasslands.

PAPER II: VERSATILITY IN FUNGI: *IN VITRO* EVIDENCE OF ECOLOGICAL TRANSITIONS IN THE GENUS *MYCENA*

The transition from saprotrophic to mycorrhizal lifestyle has occurred several times independently during evolution, suggesting a strong, and presumably still operating, evolutionary pressure for saprotrophic fungi to switch to symbiotic associations with plants. There are numerous studies reporting on saprotrophic fungi occupying healthy plant roots, blurring the borders between their predefined ecological roles. The aim of this paper was to investigate whether the ubiquitous saprotrophic genus *Mycena*, that are frequently major components of molecular studies of root-associated fungal communities, can form biotrophic interactions with plants. We selected 17 *Mycena* species for our study, covering a broad range of the genus. In order to investigate the putative interaction between *Mycena* spp. and plant roots *in vitro*, seedlings of *Betula pendula* and the 17 *Mycena* spp. were grown together in microcosms. Seedling growth was recorded, and we used cryomicrotome sectioning, differential staining and fluorescent microscope imaging to visualize fungal growth within the fine roots of the seedlings. Physiological interactions were investigated using

^{14}C and ^{32}P to look for potential transfer between seedlings and fungi. All *Mycena* spp. associated closely with fine roots, showing hyphal penetration into the roots, which in some cases were intracellular. Seven species formed mantle-like structures around root tips. *Mycena pura* and *M. galopus* both enhanced seedling growth, with *M. pura* showing transfer of significant amounts of ^{32}P to the seedlings. Our results support the view that some *Mycena* species are not purely saprotrophic and may potentially occupy a transitional state between saprotrophy and biotrophy. A genomic investigation of *Mycena* species may shed further light on evolutionary processes involved in switching to symbiosis.

PAPER III: A SINGLE ECTOMYCORRHIZAL PLANT ROOT SYSTEM INCLUDES A DIVERSE AND SPATIALLY STRUCTURED FUNGAL COMMUNITY

Although only a relatively small proportion of plant species form ectomycorrhiza (EcM) with fungi, it is crucial for growth and survival for a number of widespread woody plant species. Few studies have attempted to investigate the fine scale spatial structure of entire root systems of adult EcM plants, partly because most form very large root systems. The aim of this study was to investigate how EcM communities are structured on a very fine scale. We used the herbaceous perennial *Bistorta vivipara*, which has a small and condensed root system, to map the entire root system of an adult EcM plant. We investigate the spatial structure of its root-associated fungi, and found a strong gradient within the root system. All EcM root tips were sampled, mapped and identified using a direct PCR approach and Sanger sequencing of the ITS region. A total of 32.1% of all sampled root tips (739 of 2302) were successfully sequenced and clustered into 41 OTUs. We observed a clear spatial structuring of the root-associated fungi within the root system. Clusters of individual OTUs were observed in the younger parts of the root system, consistent with observations of *priority effect* in previous studies, but were absent from the older parts of the root system. This may suggest a succession and fragmentation of the root-associated fungi even at a very fine scale, where competition likely comes into play at different successional stages within the root system.

DISCUSSION

The papers in this thesis show how different factors are important in structuring fungal communities at different scales, and that observations from field studies can be supported by *in vitro* laboratory studies for more in-depth knowledge on fungi's ecology. Here, I will discuss the different aspects that tie the three papers together and also emphasize their main differences.

COMMUNITY COMPOSITION AND STRUCTURE

Overall, we recovered diverse fungal communities from the environmental samples in Paper I and III, although on very different scales. The fungal community recovered from a single *B. vivipara* root system included 41 OTUs, which was higher than the average number of fungal OTUs recovered from *B. vivipara* roots in Paper I (range: 11-53, mean: 22.75). Both studies fall well within the range of previous studies on root-associated fungal communities of *B. vivipara* (Blaalid *et al.*, 2012; Yao *et al.*, 2013; Botnen *et al.*, 2014). We recovered a somewhat lower number of OTUs from *P. erecta* (range 7-37, mean: 17.12) and slightly higher from soil samples (range: 9-69, mean: 23.11). In Paper III, we excluded most DNA sequences that did not have affinity to taxa that are EcM or other known root-associates. The most common OTUs in Paper III, with taxonomic affinities to genera *Pseutomentella*, *Tomentella*, *Cortinarius* and *Russula*, were also among the most common genera recovered from *B. vivipara* in Paper I.

Potentilla erecta has been recorded as both AM and as non-mycorrhizal (reviewed in Wang & Qiu, 2006), but because of our choice of primers (ITS1-F and ITS2), we recovered a very low abundance of AM fungi (1-4‰ in two *P. erecta* samples). Although not emphasized in Paper I, the most common OTUs in *P. erecta* belonged to *Mycena* spp. and *Hygrocybe* spp., both of which are genera that has been described as purely saprotrophic in the Nordic countries (Knudsen & Vesterholt, 2008). However, recent evidence points to that *Hygrocybe* spp. are most likely biotrophic (Halbwachs *et al.*, 2018). Among other common OTUs recovered from *P. erecta* roots were several OTUs with affinity to root-associated ascomycetes, such as *Cladophialophora*, *Phialocephala*, *Cadophora* and *Melinomyces*, but also a few EcM-forming genera, such as *Russula*, *Tomentella* and *Tuber*. This indicates that *P. erecta* might possess a more diverse array of mycorrhizal fungi than earlier appreciated (discussed below).

Diversity of fungi have in general been found to be higher in soils than in plant roots (Peay *et al.*, 2016). Although we recovered similar amounts of OTUs from soils and plant roots in Paper I, the fungal community in soil overlapped to a large extent with the root-associated fungal community, although with more OTUs annotated as saprotrophs in soil.

In both Paper I and III, the fungal communities were spatially structured, although the scales were vastly different (mm-cm in Paper III and extending over 100 km apart in paper I). On a very small

scale (Paper III), the root-associated fungal community seemed to be structured by competition and *priority effects*, although no other edaphic factors were measured and cannot be ruled out. The strong spatial structure within the root system was related to root threads position along the rhizome.

On larger scales, the root-associated fungal communities were structured by both temperature and precipitation along a climatic gradient in western Norway. Generally, the root-associated fungal communities were more affected by the climatic drivers compared to the fungal communities in soil. Plant communities have been found to be influenced by differences in both temperature and precipitation levels across the gradient (Klanderud *et al.*, 2015; Tingstad *et al.*, 2015), and the root-associated fungal community may be also indirectly influenced by climate through their plant hosts. However, the strongest effects by climatic factors were seen when the dataset was analysed as a whole (i.e. both plant root-associated and soil fungi). Community composition for all sample types clearly correlated to both temperature and precipitation, underpinning the importance of investigating different compartments of the belowground mycobiome in concert.

FUNGAL SPECIES, OTUS AND CLUSTERING LEVEL

Most ecological studies on animals and plants are conducted on a species level, but species-level interpretations are often more problematic in fungal ecology. In Paper II, the fungal strains included were isolated from sporocarps of morphological well-known *Mycena* species. Thus, even if there are some evidence of cryptic species diversity with *Mycena* (Harder *et al.*, 2013), the species interpretation was relatively straightforward. The identity of the cultures were also confirmed with high quality Sanger sequences of the ITS region. For environmental samples, however, where many of the DNA reads are not assigned to taxa below phylum level (Nilsson *et al.*, 2016), the picture becomes more complex, and especially so for HTS datasets. In paper I we obtained over 17 million DNA reads of the ITS1 region after quality filtering using Illumina technology, whereas in Paper III we worked with 739 Sanger sequences of the entire ITS region (ITS1, 5.8S and ITS2) after quality filtering. The lower number of DNA sequences in Paper III allowed for manual inspection and quality control, and sequences were subsequently clustered into OTUs using a 97% similarity threshold. The OTUs where then manually taxonomically assigned using species hypothesis (SH) from the fungal database UNITE (Kõljalg *et al.*, 2013). Such manual approaches are not suitable for datasets with millions of DNA reads. Hence, in Paper I, quality filtering and chimera removal was done using DADA2 (Callahan *et al.*, 2016). The output of DADA2 provides amplicon sequence variant (ASVs), which may vary by as little as one or two nucleotides. As intraspecific variation in ITS can be high (Nilsson *et al.*, 2008), ASVs are not good proxies for species in Fungi, and will inflate the richness. Because of this, we did post-clustering of the ASVs into OTUs using LULU (Frøslev *et al.*, 2017), reducing the number of units to about half the initial ASVs. Intraspecific variation in ITS varies across taxa (Nilsson *et al.*, 2008), but a sequence similarity threshold between 97-98% is generally thought to retain a balance

between intraspecific sequence variance and sequencing errors (Nilsson *et al.*, 2018). It is impossible, though, to accurately determine to what degree the OTUs we investigated in Paper I and Paper III represent real fungal species (Nilsson *et al.*, 2008; Blaalid *et al.*, 2013). However, if you are not looking for answers regarding species richness or diversity, clustering level and OTU delineation has very little influence on the overall structure of community composition, if strong underlying gradients are present (Botnen *et al.*, 2018), such as in Paper I and III.

FUNCTIONS OF BELOWGROUND FUNGAL COMMUNITIES

With large sequence-based studies, functional and ecological inference is typically done by grouping species into trophic modes and/or guilds based on existing knowledge of their nutritional mode and ecology (e.g. as done in Clemmensen *et al.*, 2006, 2015; Kyaschenko *et al.*, 2017; Sterkenburg *et al.*, 2018). Assigning fungal functions in metabarcoding studies has now become relatively easy by means of the open annotation tool FUNGuild (Nguyen *et al.*, 2016), which assigns taxonomically annotated OTUs into functional guilds. Ideally, ecological functions of fungi should be assigned on a species level, but as discussed above, OTUs may not necessarily represent biological species. Trophic strategies are often conserved within fungal genera, although there are several exceptions (Humpert *et al.*, 2001; Tulloss *et al.*, 2016). We used FUNGuild in Paper I, which assigns OTUs to functional guilds based on taxonomic annotation at genus level. In addition, we manually inspected all assigned fungal guilds. Assignments are done using a confidence ranking, from 'highly probable', 'probable' and 'possible', but for genera that include species with different ecological functions the output can be somewhat complicated. As an example, an OTU annotated to the genus *Melinomyces* was assigned to the functional guild 'Ectomycorrhizal-Endophyte-Ericoid Mycorrhizal-Litter Saprotroph-Orchid Mycorrhizal' with the confidence level 'probable'. Thus, for the majority of the fungal guilds manual revisions were necessary, and has also been done in other studies where FUNGuild was used for functional guild annotation (Kolaříková *et al.*, 2017). For several OTUs assigned to genera with consistent and well-known ecological functions, such as many EcM fungi, the output from FUNGuild did not require any further revision.

Among other groups, the genus *Mycena* that has been consistently reported as saprotrophic in Europe (e.g. Clemmensen *et al.*, 2006; Knudsen & Vesterholt, 2008; Kyaschenko *et al.*, 2017; Sterkenburg *et al.*, 2018) was significantly more abundant in both plant roots than in soil (Paper I), and the most abundant genus in *P. erecta*. The genus was also assigned to the 'Pathotroph-Saprotroph' by FUNGuild. However, we show in Paper II that members within the genus *Mycena* readily forms associations with roots of *B. pendula* seedlings *in vitro*, which suggests that Mycena can also be involvued in biotrophic associations.

In Paper III, we focused on known root-associated species, and inclusion of OTUs was based on literature search, which is not a large effort for 41 OTUs. Intrinsically, when looking at plant roots, one would assume that the recovered fungal community is root-associated, but often OTUs with affinity to saprotrophic fungi are assumed to be contaminants (e.g. Liao *et al.*, 2014, see also discussion in Selosse *et al.*, 2010, 2018;). However, we did include a few OTUs that did not have affinity to known EcM or root-associated fungal species. An OTU with a 100% sequence similarity to the *Sarcoleotia globosa* SH in the UNITE database, was included, although never reported from plant roots before. *Sarcoleotia globosa* forms small, inconspicuous sporocarps, and has its major distribution in arctic and sub-arctic regions (Schumacher & Sivertsen, 1987; Jumpponen *et al.*, 1997), and we deemed it very unlikely that this could be contamination. Isotopic signature of sporocarps may indicate trophic status (Hobbie *et al.*, 1999). Interestingly, several members within Geoglossales have a relatively high $\delta^{15}N$ values (Griffith *et al.*, 2002), which may be indicative of a biotrophic lifestyle (Halbwachs *et al.*, 2018). Even with a relatively small dataset as in Paper III, assignment of functions is not straightforward.

Although *P. erecta* is sometimes reported as AM, we used primers that do not perform well with AM fungi in Paper I. Interestingly, in the plant roots of *P. erecta*, a large proportion of sequence reads belonged to known EcM genera, such as *Russula*, *Tomentella* and *Tuber*. Recently, it has been shown that the EcM forming species *Tuber melanosporum* occupy the roots of AM plants as endophytes (Schneider-Maunoury *et al.*, 2018), and a possible explanation may be that these genera similarly occupy the roots of *P. erecta* as endophytes. We would probably not have recovered these fungal genera with AM-specific primers (e.g. Kohout *et al.*, 2014).

A more direct way of looking at fungal functions in environmental communities, is to investigate fungal enzymatic activities (e.g. Kyaschenko *et al.*, 2017). In Paper I, we included enzyme assays of fungal hydrolytic enzymes, to see whether changes in enzyme activity could be related to the fungal community and climatic variables. We observed that enzyme activity increased with increased fungal biomass (measured as ergosterol content). This observation does not explain specific functions of specific fungal taxa, only that more enzymatic activity is measured when more fungal biomass is present. However, when standardizing for the amount of fungal biomass, we were able to resolve, to some degree, how these enzymatic activities were distributed along the climatic gradient, and how they relate to fungal genera. Generally, fungal genera related to lower enzymatic activity and higher amounts of fungal biomass were the EcM genera *Russula* and *Amphinema*, as well as the root-associated ascomycetes *Cladophialophora* and *Cadphora*.

Root-associated ascomycetes (i.e. dark septate endophytes) have received relatively little attention compared to EcM fungi (but see for instance Trappe & Jumpponen, 1998; Tedersoo *et al.*, 2009), and

Photo: Ella Thoen

"When observing nature, we often see what we are specifically looking for: this may be one of the reasons why our understanding of fungal niches is long-standing, but not accurate."

(Selosse *et al.*, 2018, p. 969)

their functions remain largely unknown. Melanized root-associated ascomycetes, such as *Cladophialophora*, *Exophiala* and *Cadophora* were among the more abundant genera in drier sites in Paper I, which were also the sites with highest fungal biomass and soil carbon content. Melanin has been shown to be recalcitrant to decomposition (Fernandez *et al.*, 2016; Fernandez & Kennedy, 2018), and thus the melanized root-associated ascomycetes may be related to higher soil carbon content in these sites. Previous studies have emphasized the role of mycorrhizal fungi in in soil carbon dynamics (Clemmensen *et al.*, 2013; Averill *et al.*, 2014; Averill & Hawkes, 2016; Hagenbo *et al.*, 2017), but our findings suggest that this group may possible be important in soil carbon dynamics in semi-natural grasslands. Thus, in the light of climate change and global carbon dynamics, perhaps these deserve more attention.

SAPROTROPHY, BIOTROPHY OR MUTUALISM?

The strict divisions between ecological roles of fungi are longstanding, but currently the borders between previously defined ecological groups are being challenged. Mycorrhizal fungi have retained a repertoire of genes related to decomposition (Martino *et al.*, 2018; Op De Beeck *et al.*, 2018), species defined as strict plant pathogens can act also as harmless endophytes in other plant species (Lofgren *et al.*, 2018) and well-studied EcM species are showing up in the roots of non-EcM plants (Gryndler *et al.*, 2014; Schneider-Maunoury *et al.*, 2018). Thus, ecological niches in fungi seem to be more flexible than previously believed, which we also find support for of in the three papers in this thesis. In Paper III, several of the detected fungi in EcM root tips did not belong to known root-associated taxa, and a few, we deemed unlikely to be contaminants. Thus, we opened for the possibility that they could be biotrophically associated with plant-roots. In Paper I, a large proportion of the OTUs recovered from plant roots belonged to assumed saprotrophic fungi, and the most abundant genus in *P. erecta* was *Mycena*. Alongside known EcM genera, *Mycena* was also among the most abundant genera in *B. vivipara* roots.

In Paper II we investigated the relationship between a selection of *Mycena* spp. and *B. pendula* roots. Several studies point to that several members of this genus may be biotrophic (Zhang *et al.*, 2012; Lorberau *et al.*, 2017; Smith *et al.*, 2017). Some *Mycena* spp. have been documented as mycorrhizas in Asian *Dendrobium* orchids (Zhang *et al.*, 2012), and *M. galopus* enhanced growth of *Vaccinium corumbosum* seedlings (Grelet *et al.*, 2017). In Paper II we document that *Mycena* spp. are indeed capable of root colonization all the way into the vascular tissues of living seedlings, and even nutrient transfer in a few cases.

It is likely that several root-associated fungi exists somewhere along the saprotrophy-biotrophy continuum, as has been discussed for EcM fungi (Koide *et al.*, 2008). Because mycorrhizal species have evolved from saprotrophic ancestors (Tedersoo *et al.*, 2010; Wolfe *et al*, 2012; Kohler *et al.*,

2015), and evolution is constantly ongoing, it is reasonable to believe that several species are in transitional states between saprotrophy and biotrophy. Vice versa, several saprotrophic species are capable of ensheating root-tips, forming mantle-like structures (Vasiliauskas *et al.*, 2007; Eastwood *et al.*, 2011; Smith *et al.*, 2017), similar to those formed by EcM fungi. In a comprehensive survey of 201 wood-decaying basidiomycetes, almost 17% of the investigated species formed facultative biotrophic interactions with plant roots *in vitro* (Smith *et al.*, 2017). As a commentary to the Smith *et al.* (2017) study, Baldrian & Kohout (2017) discuss the possibility of observing emergence of novel EcM fungi operating in dual niches. Reporting on all taxa recovered from HTS studies, and not only the functional guild of interest, is therefore encouraged (Selosse *et al.*, 2010, 2018; Baldrian & Kohout, 2017)

CONCLUSIONS AND FUTURE PERSPECTIVES

In the largely unseen and cryptic world of belowground fungi, generalisations into 'boxes' of functional groups and ordering of DNA sequences into OTUs are practical and also necessary to make ecological inferences. These pragmatic 'boxes' are needed to understand and communicate ecological processes in the complex systems of belowground fungal communities, where we still know relatively little about drivers, interactions and functions of the communities. Fungal ecology is to a large degree still an exploratory field, and in the more explorative parts of fungal ecology, one might be more open-minded about tentative findings and relationships. When trying to squeeze this complex world into a black-white hypothesis testing world, there is always a risk of excluding relevant factors, especially in poorly studied systems.

The tools for fungal ecologists for sorting their HTS data are numerous and continuously improving (Kõljalg *et al.*, 2013; Callahan *et al.*, 2016; Nguyen *et al.*, 2016; Frøslev *et al.*, 2017), and our knowledge of diversity, distribution, environmental drivers and functions of fungal communities have vastly improved since the 'molecular revolution' in fungal ecology (reviewed in Peay *et al.*, 2016). We should strive to continue to improve our understanding, and develop knowledge, tools and databases that will be easily available for other fungal ecologists to implement (such as FUNGuild; Nguyen *et al.*, 2016 and UNITE; Kõljalg *et al.*, 2013).

Although HTS studies have become the gold standard in fungal ecology (Lindahl *et al.*, 2013; Nilsson *et al.*, 2018), the more classical methods, such as Sanger sequencing or *in vitro* experiments, still provide valuable additions to our growing knowledge. The genus *Mycena* was frequently recovered from plant roots in molecular based studies (Bougoure *et al.*, 2007; Blaalid *et al.*, 2012; Botnen *et al.*, 2014; Lorberau *et al.*, 2017), but the function of these fungi in roots remained unexplained. By *in vitro* experiments, we showed that *Mycena* species were able to colonize roots, and even transferring nutrients to *B. pendula* seedlings. Using radioactive isotopes for tracing nutrient exchange was

implemented by Melin & Nilsson as early as 1950 to investigate nutrient exchange between plants and mycorrhizal fungi (Melin & Nilsson, 1950). 'Old-fashioned' methods still provide useful information, and I argue that looking to the past as well as the future, may aid us in our search for deeper knowledge about fungal ecology.

One major challenge in our progress in understanding fungal ecology is that many scientist often only look at what they set out to look at (discussed in Selosse *et al.*, 2018). As an example, lichens were until recently believed to be a symbiosis between a single fungal partner (usually an ascomycete) and photosynthesizing partner(s). Thus, lichenologists used primers designed for Ascomycota in molecular studies. Spribille *et al.* (2016) has now showed that specific basidiomycete yeasts are common in the cortex of several macro-lichens, and in some cases basidiomycete yeasts were the only differences between described lichen morpho-species. This had remained undiscovered for decades after the 'molecular revolution', because lichenologists were not looking for basidiomycetes. Similarly, the resent discovery that the EcM forming ascomycete *T. melanosporum* may occupy roots of non-EcM plants (Gryndler *et al.*, 2014; Schneider-Maunoury *et al.*, 2018), was probably not discovered before, since primers for AM fungi does not amplify ascomycetes well. As our knowledge grows, predefined niches and functions of fungi are being challenged. It is time for us to broaden our perspective, and also look outside our own 'box' of interest, to be able to answer bigger questions in fungal ecology.

ACKNOWLEDGMENTS

I would like to thank Håvard Kauserud, Unni Vik and Inger Skrede for valuable inputs on the drafts of this introduction, and Erlend Y. Fines for figure drawings.

REFERENCES

Anderson JB, Bruhn JN, Kasimer D, Wang H, Rodrigue N, Smith ML. **2018**. Clonal evolution and genome stability in a 2,500-year-old fungal individual. *bioRxiv*: 377234.

Andrew C, Halvorsen R, Heegaard E, Kuyper TW, Heilmann-Clausen J, Krisai-Greilhuber I, Bässler C, Egli S, Gange AC, Høiland K, *et al.* 2018. Continental-scale macrofungal assemblage patterns correlate with climate, soil carbon and nitrogen deposition. *Journal of Biogeography* **45**: 1942–1953.

Averill C, Hawkes C V. 2016. Ectomycorrhizal fungi slow soil carbon cycling. *Ecology Letters* **19**: 937–947.

Averill C, Turner BL, Finzi AC. **2014**. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature* **505**: 543–545.

Bahram M, Hildebrand F, Forslund SK, Anderson JL, Soudzilovskaia NA, Bodegom PM, Bengtsson-Palme J, Anslan S, Coelho LP, Harend H, *et al.* 2018. Structure and function of the global topsoil microbiome. *Nature* **560**: 233–237.

Bahram M, Põlme S, Kõljalg U, Tedersoo L. **2011**. A single European aspen (*Populus tremula*) tree individual may potentially harbour dozens of *Cenococcum geophilum* ITS genotypes and hundreds of species of ectomycorrhizal fungi. *FEMS Microbiology Ecology* **75**: 313–320.

Baldrian P. **2016**. Forest microbiome: diversity, complexity and dynamics. *FEMS Microbiology Reviews* **41**: 109–130.

Baldrian P, Kohout P. **2017**. Interactions of saprotrophic fungi with tree roots: can we observe the emergence of novel ectomycorrhizal fungi? *New Phytologist* **215**: 511–513.

Baldrian P, Kolařík M, Štursová M, Kopecký J, Valášková V, Větrovský T, Žifčáková L, Šnajdr J, Rídl J, Vlček Č, *et al.* 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *The ISME Journal* **6**: 248–258.

Baldrian P, Valášková V. **2008**. Degradation of cellulose by basidiomycetous fungi. *FEMS Microbiology Reviews* **32**: 501–521.

Op De Beeck M, Troein C, Peterson C, Persson P, Tunlid A. **2018**. Fenton reaction facilitates organic nitrogen acquisition by an ectomycorrhizal fungus. *New Phytologist* **218**: 335–343.

Begerow D, Nilsson H, Unterseher M, Maier W. **2010**. Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology* **87**: 99–108.

Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H. **2010**. ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. *BMC Microbiology* **10**: 189.

Bjørnsgaard Aas A, Davey ML, Kauserud H. **2017**. ITS all right mama: investigating the formation of chimeric sequences in the ITS2 region by DNA metabarcoding analyses of fungal mock communities of different complexities. *Molecular Ecology Resources* **17**: 730–741.

Blaalid R, Carlsen T, Kumar S, Halvorsen R, Ugland KI, Fontana G, Kauserud H. **2012**. Changes in the root-associated fungal communities along a primary succession gradient analysed by 454 pyrosequencing. *Molecular Ecology* **21**: 1897–1908.

Blaalid R, Davey ML, Kauserud H, Carlsen T, Halvorsen R, Høiland K, Eidesen PB. **2014**. Arctic root-associated fungal community composition reflects environmental filtering. *Molecular Ecology* **23**: 649–659.

Blaalid R, Kumar S, Nilsson RH, Abarenkov K, Kirk PM, Kauserud H. **2013**. ITS1 versus ITS2 as DNA metabarcodes for fungi. *Molecular Ecology Resources* **13**: 218–224.

Blackwell M. **2011**. The fungi: 1, 2, 3 ... 5.1 million species? *American Journal of Botany* **98**: 426–438.

Bogar LM, Peay KG. **2017**. Processes Maintaining the Coexistence of Ectomycorrhizal Fungi at a Fine Spatial Scale. In: Tedersoo L, ed. Biogeography of Mycorrhizal Symbiosis. Cham: Springer International Publishing, 79–105.

Botnen SS, Davey ML, Halvorsen R, Kauserud H. **2018**. Sequence clustering threshold has little effect on the recovery of microbial community structure. *Molecular Ecology Resources* **18**: 1064–1076.

Botnen S, Vik U, Carlsen T, Eidesen PB, Davey ML, Kauserud H. **2014**. Low host specificity of root-associated fungi at an Arctic site. *Molecular Ecology* **23**: 975–985.

Bougoure DS, Parikin PI, Cairney JWG, Alexander IJ, Anderson IC. **2007**. Diversity of fungi in hair roots of Ericaceae varies along a vegetation gradient. *Molecular Ecology* **16**: 4624–4636.

Brundrett M. **2006**. Understanding the Roles of Multifunctional Mycorrhizal and Endophytic Fungi. In: (Schulz BJE, Boyle CJC, Sieber TN, eds.) Microbial Root Endophytes. Berlin, Heidelberg: Springer Berlin Heidelberg, 282–298.

Brundrett MC, Tedersoo L. **2018**. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist* **220**: 1108–1115.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. **2016**. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Ceccaroli P, Saltarelli R, Polidori E, Barbieri E, Guescini M, Ciacci C, Stocchi V. **2015**. Sugar transporters in the black truffle *Tuber melanosporum*: From gene prediction to functional characterization. *Fungal Genetics and Biology* **81**: 52–61.

Clemmensen KE, Bahr A, Ovaskainen O, Dahlberg A, Ekblad A, Wallander H, Stenlid J, Finlay RD, Wardle DA, Lindahl BD. **2013**. Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* **339**: 1615–1618.

Clemmensen KE, Finlay RD, Dahlberg A, Stenlid J, Wardle DA, Lindahl BD. **2015**. Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist* **205**: 1525–1536.

Clemmensen KE, Michelsen A. **2006**. Integrated long-term responses of an arctic–alpine willow and associated ectomycorrhizal fungi to an altered environment. *Canadian Journal of Botany* **84**: 831–843.

Clemmensen KE, Michelsen A, Jonasson S, Shaver GR. **2006**. Increased ectomycorrhizal fungal abundance after long-term fertilization and warming of two arctic tundra ecosystems. *New Phytologist* **171**: 391–404.

Crowther TW, Todd-Brown KEO, Rowe CW, Wieder WR, Carey JC, Machmuller MB, Snoek BL, Fang S, Zhou G, Allison SD, *et al.* 2016. Quantifying global soil carbon losses in response to warming. *Nature* **540**: 104–108.

Daft MJ, Nicolson TH. **1966**. Effect of endogone mycorrhiza on plant growth. *New Phytologist* **65**: 343–350.

Dahlberg A. **2001**. Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. *New Phytologist* **150**: 555–562.

Delaux P-M, Séjalon-Delmas N, Bécard G, Ané J-M. **2013**. Evolution of the plant–microbe symbiotic 'toolkit'. *Trends in Plant Science*: 12–18.

Eastwood DC, Floudas D, Binder M, Majcherczyk A, Schneider P, Aerts A, Asiegbu FO, Baker SE, Barry K, Bendiksby M, *et al.* 2011. The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. *Science* **333**: 762–765.

Fernandez CW, Kennedy PG. **2018**. Melanization of mycorrhizal fungal necromass structures microbial decomposer communities. *Journal of Ecology* **106**: 468–479.

Fernandez CW, Langley JA, Chapman S, McCormack ML, Koide RT. **2016**. The decomposition of ectomycorrhizal fungal necromass. *Soil Biology and Biochemistry* **93**: 38–49.

Frøslev TG, Kjøller R, Bruun HH, Ejrnæs R, Brunbjerg AK, Pietroni C, Hansen AJ. **2017**. Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nature Communications* **8**: 1188.

Gardes M, Bruns TD. **1993**. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.

Grelet GA, Ba R, Goeke DF, Houliston GJ, Taylor AFS, Durall DM. **2017**. A plant growth-promoting symbiosis between *Mycena galopus* and *Vaccinium corymbosum* seedlings. *Mycorrhiza* **27**: 831–839.

Griffith GW, Easton GL, Jones AW. **2002**. Ecology and diversity of waxcap (*Hygrocybe* spp.) Fungi. *Botanical Journal of Scotland* **54**: 7–22.

Gryndler M, Černá L, Bukovská P, Hršelová H, Jansa J. **2014**. *Tuber aestivum* association with non-host roots. *Mycorrhiza* **24**: 603–610.

Hagenbo A, Clemmensen KE, Finlay RD, Kyaschenko J, Lindahl BD, Fransson P, Ekblad A. **2017**. Changes in turnover rather than production regulate biomass of ectomycorrhizal fungal mycelium across a *Pinus sylvestris* chronosequence. *New Phytologist* **214**: 424–431.

Halbwachs H, Easton GL, Bol R, Hobbie EA, Garnett MH, Peršoh D, Dixon L, Ostle N, Karasch P, Griffith GW. **2018**. Isotopic evidence of biotrophy and unusual nitrogen nutrition in soil-dwelling Hygrophoraceae. *Environmental Microbiology* **20**: 3573–3588.

Harder CB, Læssøe T, Frøslev TG, Ekelund F, Rosendahl S, Kjøller R. **2013**. A three-gene phylogeny of the *Mycena pura* complex reveals 11 phylogenetic species and shows ITS to be unreliable for species identification. *Fungal Biology* **117**: 764–775.

van der Heijden MGA, Martin FM, Selosse M-A, Sanders IR. **2015**. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist* **205**: 1406–1423.

Hiscox J, Savoury M, Müller CT, Lindahl BD, Rogers HJ, Boddy L. **2015**. Priority effects during fungal community establishment in beech wood. *The ISME Journal* **9**: 2246–2260.

Hobbie EA, Macko SA, Shugart HH. **1999**. Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. *Oecologia* **118**: 353.

Horton TR, Bruns TD. **2001**. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* **10**: 1855–1871.

Humpert AJ, Muench EL, Giachini AJ, Castellano MA, Spatafora JW. **2001**. Molecular phylogenetics of *Ramaria* and related genera: Evidence from nuclear large subunit and mitochondrial small subunit rDNA sequences. *Mycologia* **93**: 465–477.

Jarvis S, Woodward S, Alexander IJJ, Taylor ASF. **2013**. Regional scale gradients of climate and nitrogen deposition drive variation in ectomycorrhizal fungal communities associated with native Scots pine. *Global Change Biology* **19**: 1688–1696.

Jumpponen A, Weber NS, Trappe JM, Cázares E. **1997**. Distribution and ecology of the ascomycete *Sarcoleotia globosa* in the United States. *Canadian Journal of Botany* **75**: 2228–2231.

Kasurinen A, Koikkalainen K, Anttonen MJ, Possen B, Oksanen E, Rousi M, Vapaavuori E, Holopainen T. **2016**. Root morphology, mycorrhizal roots and extramatrical mycelium growth in silver birch (*Betula pendula* Roth) genotypes exposed to experimental warming and soil moisture manipulations. *Plant and Soil* **407**: 341–353.

Kauserud H, Kumar S, Brysting AK, Nordén J, Carlsen T. **2012**. High consistency between replicate 454 pyrosequencing analyses of ectomycorrhizal plant root samples. *Mycorrhiza* **22**: 309–315.

Kennedy PG, Bruns TD. **2005**. Priority effects determine the outcome of ectomycorrhizal competition between two *Rhizopogon* species colonizing *Pinus muricata* seedlings. *New Phytologist* **166**: 631–638.

Kennedy PG, Peay KG, Bruns TD. **2009**. Root tip competition among ectomycorrhizal fungi: Are priority effects a rule or an exception? *Ecology* **90**: 2098–2107.

Kjøller R. **2006**. Disproportionate abundance between ectomycorrhizal root tips and their associated mycelia. *FEMS Microbiology Ecology* **58**: 214–224.

Klanderud K, Vandvik V, Goldberg D. **2015**. The importance of biotic vs. abiotic drivers of local plant community composition along regional bioclimatic gradients. *PLOS ONE* **10**: e0130205.

Knudsen H, Vesterholt J. **2008**. *Funga Nordica*. Copenhagen, Denmark: Nordsvamp.

Kohler A, Kuo A, Nagy LG, Morin E, Barry KW, Buscot F, Canbäck B, Choi C, Cichocki N, Clum A, *et al.* 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics* **47**: 410–415.

Kohout P, Charvátová M, Štursová M, Mašínová T, Tomšovský M, Baldrian P. **2018**. Clearcutting alters decomposition processes and initiates complex restructuring of fungal communities in soil and tree roots. *The ISME Journal* **12**: 692–703.

Kohout P, Sudová R, Janoušková M, Čtvrtlíková M, Hejda M, Pánková H, Slavíková R, Štajerová K, Vosátka M, Sýkorová Z. **2014**. Comparison of commonly used primer sets for evaluating arbuscular mycorrhizal fungal communities: Is there a universal solution? *Soil Biology and Biochemistry* **68**: 482–493.

Koide RT, Fernandez C, Malcolm G. **2014**. Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. *New Phytologist* **201**: 433–439.

Koide RT, Sharda JN, Herr JR, Malcolm GM. **2008**. Ectomycorrhizal fungi and the biotrophy–saprotrophy continuum. *New Phytologist* **178**: 230–233.

Kolaříková Z, Kohout P, Krüger C, Janoušková M, Mrnka L, Rydlová J. **2017**. Root-associated fungal communities along a primary succession on a mine spoil: Distinct ecological guilds assemble differently. *Soil Biology and Biochemistry* **113**: 143–152.

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, *et al.* 2013. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* **22**: 5271–5277.

Kyaschenko J, Clemmensen KE, Hagenbo A, Karltun E, Lindahl BD. **2017**. Shift in fungal communities and associated enzyme activities along an age gradient of managed *Pinus sylvestris* stands. *The ISME Journal* **11**: 863–874.

Laiho O. **1970**. *Paxillus involutus* as a mycorrhizal symbiont of forest trees. *Acta Forestalia Fennica* **106**: 1–78.

Liao H-L, Chen Y, Bruns TD, Peay KG, Taylor JW, Branco S, Talbot JM, Vilgalys R. **2014**. Metatranscriptomic analysis of ectomycorrhizal roots reveals genes associated with *Piloderma–Pinus* symbiosis: improved methodologies for assessing gene expression in situ. *Environmental Microbiology* **16**: 3730–3742.

Lilleskov E a, Bruns TD, Horton TR, Taylor D, Grogan P. **2004**. Detection of forest stand-level spatial structure in ectomycorrhizal fungal communities. *FEMS Microbiology Ecology* **49**: 319–32.

Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K, Carlsen T, Kjøller R, Kõljalg U, Pennanen T, Rosendahl S, Stenlid J, *et al.* 2013. Fungal community analysis by high-throughput sequencing of amplified markers - a user's guide. *New Phytologist* **199**: 288–299.

Lofgren LA, LeBlanc NR, Certano AK, Nachtigall J, LaBine KM, Riddle J, Broz K, Dong Y, Bethan B, Kafer CW, *et al.* 2018. *Fusarium graminearum*: pathogen or endophyte of North American grasses? *New Phytologist* **217**: 1203–1212.

Lorberau KE, Botnen SS, Mundra S, Aas AB, Rozema J, Eidesen PB, Kauserud H. **2017**. Does warming by open-top chambers induce change in the root-associated fungal community of the arctic dwarf shrub *Cassiope tetragona* (Ericaceae)? *Mycorrhiza* **27**: 513–524.

Martino E, Morin E, Grelet GA, Kuo A, Kohler A, Daghino S, Barry KW, Cichocki N, Clum A, Dockter RB, *et al.* 2018. Comparative genomics and transcriptomics depict ericoid mycorrhizal fungi as versatile saprotrophs and plant mutualists. *New Phytologist* **217**: 1213–1229.

Massicotte HB, Melville LH, Peterson RL, Luoma DL. **1998**. Anatomical aspects of field ectomycorrhizas on *Polygonum viviparum* (Polygonaceae) and *Kobresia bellardii* (Cyperaceae). *Mycorrhiza* **7**: 287–292.

Melin E, Nilsson H. **1950**. Transfer of radioactive phosphorus to pine seedlings by means of mycorrhizal hyphae. *Physiologia Plantarum* **3**: 88–92.

Melin E, Nilsson H, Hasckaylo E. **1958**. Translocation of cations to seedlings of *Pinus virginiana* through mycorrhizal mycelium. *Botanical Gazette* **119**: 243–246.

Menkis A, Vasiliauskas R, Taylor AFS, Stenlid J, Finlay R. **2005**. Fungal communities in mycorrhizal roots of conifer seedlings in forest nurseries under different cultivation systems, assessed by morphotyping, direct sequencing and mycelial isolation. *Mycorrhiza* **16**: 33–41.

Miyamoto Y, Sakai A, Hattori M, Nara K. **2015**. Strong effect of climate on ectomycorrhizal fungal composition: evidence from range overlap between two mountains. *The ISME Journal* **9**: 1870–1879.

Mundra S, Halvorsen R, Kauserud H, Müller E, Vik U, Eidesen PB. **2015**. Arctic fungal

communities associated with roots of *Bistorta vivipara* do not respond to the same fine-scale edaphic gradients as the aboveground vegetation. *New Phytologist* **205**: 1587–1597.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. **2016**. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241–248.

Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L. **2018**. Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nature Reviews Microbiology*.

Nilsson R, Kristiansson E, Ryberg M, Hallenberg N, Larsson1 K-H. **2008**. Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary Bioinformatics* **4**: 193–201.

Nilsson RH, Wurzbacher C, Bahram M, R. M. Coimbra V, Larsson E, Tedersoo L, Eriksson J, Duarte C, Svantesson S, Sánchez-García M, *et al.* 2016. Top 50 most wanted fungi. *MycoKeys* **12**: 29–40.

O'Brien HE, Parrent JLJ, Jackson JA, Moncalvo JJ-M, Vilgalys R. **2005**. Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology* **71**: 5544–5550.

Peay KG, Kennedy PG, Talbot JM. **2016**. Dimensions of biodiversity in the Earth mycobiome. *Nature Reviews Microbiology* **14**: 434–447.

Pena R, Offermann C, Simon J, Naumann PS, Geßler A, Holst J, Dannenmann M, Mayer H, Kögel-Knabner I, Rennenberg H, *et al.* 2010. Girdling affects ectomycorrhizal fungal (EMF) diversity and reveals functional differences in EMF community composition in a beech forest. *Applied and Environmental Microbiology* **76**: 1831–1841.

Pickles BJ, Genney DR, Anderson IC, Alexander IJ. **2012**. Spatial analysis of ectomycorrhizal fungi reveals that root tip communities are structured by competitive interactions. *Molecular Ecology* **21**: 5110–5123.

Le Quéré A, Wright DP, Söderström B, Tunlid A, Johansson T. **2005**. Global patterns of gene regulation associated with the development of ectomycorrhiza between birch (*Betula pendula* Roth.) and *Paxillus involutus* (Batsch) Fr. *Molecular Plant-Microbe Interactions* **18**: 659–673.

Redecker D, Kodner R, Graham LE. **2000**. Glomalean fungi from the Ordovician. *Science* **289**: 1920–1921.

Reuter JA, Spacek D V., Snyder MP. **2015**. High-throughput sequencing technologies. *Molecular Cell* **58**: 586–597.

Rodriguez RJ, White Jr JF, Arnold AE, Redman RS. **2009**. Fungal endophytes: diversity and functional roles. *New Phytologist* **182**: 314–330.

Rosling A, Cox F, Cruz-Martinez K, Ihrmark K, Grelet G-A, Lindahl BD, Menkis A, James TY. **2011**. Archaeorhizomycetes: Unearthing an ancient class of ubiquitous soil fungi. *Science* **333**: 876–879.

Sanger F, Coulson AR. **1975**. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of Molecular Biology* **94**: 441–448.

Schadt CW, Martin AP, Lipson D a, Schmidt SK. **2003**. Seasonal dynamics of previously unknown fungal lineages in tundra soils. *Science* **301**: 1359–61.

Schlegel M, Münsterkötter M, Güldener U, Bruggmann R, Duò A, Hainaut M, Henrissat B, Sieber CMK, Hoffmeister D, Grünig CR. **2016**. Globally distributed root endophyte *Phialocephala subalpina* links pathogenic and saprophytic lifestyles. *BMC Genomics* **17**: 1–22.

Schneider-Maunoury L, Leclercq S, Clément C, Covès H, Lambourdière J, Sauve M, Richard F, Selosse M-AA, Taschen E. **2018**. Is *Tuber melanosporum* colonizing the roots of herbaceous, non-ectomycorrhizal plants? *Fungal Ecology* **31**: 59–68.

Schoch CL, Seifert K a, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W. **2012**. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 6241–6.

Schumacher T, Sivertsen S. **1987**. *Sarcoleotia globosa* (Sommerf.: Fr.) Korf, taxonomy, ecology and distribution. In: Laursen GA, Ammirati JF, Redhead SA, eds. *Arctic and Alpine Mycology II*. Plenum Press, New York, USA and London, UK, 163–176.

Selosse M-A, Dubois M-P, Alvarez N. **2009**. Do Sebacinales commonly associate with plant roots as endophytes? *Mycological Research* **113**: 1062–1069.

Selosse MA, Martos F, Perry B, Maj P, Roy M, Pailler T. **2010**. Saprotrophic fungal symbionts in tropical achlorophyllous orchids. *Plant Signaling & Behavior* **5**: 349–353.

Selosse M-A, Schneider-Maunoury L, Martos F. **2018**. Time to re-think fungal ecology? Fungal ecological niches are often prejudged. *New Phytologist* **217**: 968–972.

Smith ML, Bruhn JN, Anderson JB. **1992**. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. *Nature* **356**: 428–431.

Smith GR, Finlay RD, Stenlid J, Vasaitis R, Menkis A. **2017**. Growing evidence for facultative biotrophy in saprotrophic fungi: data from microcosm tests with 201 species of wood-decay basidiomycetes. *New Phytologist* **215**: 747–755.

Smith SE, Read D. **2008**. *Mycorrhizal Symbiosis*. London, UK: Elsevier Ltd.

Soudzilovskaia NA, Bodegom PM van, Moreno CT, Zelfde M van't, McCallum I, Fisher JB, McCormack LM, Brundrett M, Sa NC de, Tedersoo L, *et al.* 2018. Global mycorrhizal plants distribution linked to terrestrial carbon stocks. *bioRxiv* **49**: 331884.

Spribille T, Tuovinen V, Resl P, Vanderpool D, Wolinski H, Aime MC, Schneider K, Stabentheiner E, Toome-Heller M, Thor G, *et al.* 2016. Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* **353**: 488–492.

Sterkenburg E, Clemmensen KE, Ekblad A, Finlay RD, Lindahl BD. **2018**. Contrasting effects of ectomycorrhizal fungi on early and late stage decomposition in a boreal forest. *The ISME Journal* **12**: 2187–2197.

Strullu-Derrien C, Selosse M-A, Kenrick P, Martin FM. **2018**. The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. *New Phytologist* **220**: 1012–1030.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC. **2000**. Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* **31**: 21–32.

Tedersoo L, Bahram M, Polme S, Koljalg U, Yorou NS, Wijesundera R, Ruiz L V., Vasco-Palacios AM, Thu PQ, Suija A, *et al.* 2014. Global diversity and geography of soil fungi. *Science* **346**: 1256688–1256688.

Tedersoo L, Hallenberg N, Larsson K. **2003**. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. *New Phytologist* **159**: 153–165.

Tedersoo L, Lindahl B. **2016**. Fungal identification biases in microbiome projects. *Environmental Microbiology Reports* **8**: 774–779.

Tedersoo L, May TW, Smith ME. **2010**. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza* **20**: 217–63.

Tedersoo L, Pärtel K, Jairus T, Gates G, Põldmaa K, Tamm H. **2009**. Ascomycetes associated with ectomycorrhizas: Molecular diversity and ecology with particular reference to the Helotiales. *Environmental Microbiology* **11**: 3166–3178.

Tedersoo L, Suvi T, Jairus T, Kõljalg U. **2008**. Forest microsite effects on community composition of ectomycorrhizal fungi on seedlings of *Picea abies* and *Betula pendula*. *Environmental Microbiology* **10**: 1189–1201.

Tingstad L, Olsen SL, Klanderud K, Vandvik V, Ohlson M. **2015**. Temperature, precipitation and biotic interactions as determinants of tree seedling recruitment across the tree line ecotone. *Oecologia* **179**: 599–608.

Toju H, Sato H, Yamamoto S, Kadowaki K, Tanabe AS, Yazawa S, Nishimura O, Agata K. **2013**. How are plant and fungal communities linked to each other in belowground ecosystems? A massively parallel pyrosequencing analysis of the association specificity of root-associated fungi and their host plants. *Ecology and Evolution* **3**: 3112–3124.

Trappe JM, Jumpponen A. **1998**. Dark septate endophytes: A review of facultative biotrophic root-colonizing fungi. *New Phytologist* **140**: 295–310.

Tulloss RE, Kuyper TW, Vellinga EC, Yang ZL, Halling RE, Geml J, Sánchez-Ramírez S, Goncalves SC, Hess J, Pringle A. **2016**. The genus Amanita should not be split. *Amanitaceae* **1**: 1–16.

Vasiliauskas R, Menkis A, Finlay RD, Stenlid J. **2007**. Wood-decay fungi in fine living roots of conifer seedlings. *New Phytologist* **174**: 441–446.

Vilgalys R, Sun BL. **1994**. Ancient and recent patterns of geographic speciation in the oyster mushroom Pleurotus revealed by phylogenetic analysis of ribosomal DNA sequences. *Proceedings of the National Academy of Sciences* **91**: 4599–4603.

Voříšková J, Brabcová V, Cajthaml T, Baldrian P. **2014**. Seasonal dynamics of fungal communities in a temperate oak forest soil. *New Phytologist* **201**: 269–78.

Vrålstad T, Holst-Jensen A, Schumacher T. **1998**. The postfire discomycete *Geopyxis carbonaria* (Ascomycota) is a biotrophic root associate with Norway spruce (*Picea abies)* in nature. *Molecular Ecology* **7**: 609–616.

Wang B, Qiu Y-L. **2006**. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16**: 299–363.

Wang B, Yeun LH, Xue J-YY, Liu Y, Ané J-MM, Qiu Y-LL. **2010**. Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. *New Phytologist* **186**: 514–525.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninski JJ, White TJ eds. *PCR*

Protocols: A Guide to Methods and Applications. San Diego, USA: Academic press**,** 315-322

Wolfe BE, Tulloss RE, Pringle A. **2012**. The irreversible loss of a decomposition pathway marks the single origin of an ectomycorrhizal symbiosis. *PLoS ONE* **7**: e39597.

Yao F, Vik U, Brysting AK, Carlsen T, Halvorsen R, Kauserud H. **2013**. Substantial compositional turnover of fungal communities in an alpine ridge-to-snowbed gradient. *Molecular Ecology* **22**: 5040–52.

Yoshida N, Son J a, Matsushita N, Iwamoto K, Hogetsu T. **2014**. Fine-scale distribution of ectomycorrhizal fungi colonizing *Tsuga diversifolia* seedlings growing on rocks in a subalpine *Abies veitchii* forest. *Mycorrhiza* **24**: 247–57.

Zhang L, Chen J, Lv Y, Gao C, Guo S. **2012**. *Mycena* sp., a mycorrhizal fungus of the orchid *Dendrobium officinale*. *Mycological Progress* **11**: 395–401.