Richness and community structure of High Arctic fungi through space and time explored using high-throughput sequencing

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“I would like to dedicate this thesis to
my beloved Grandmother
and parents”

Preface

In everyone's life there seems a time when wishes do come true. I am about to finish the journey of my PhD and there are no words to express the feelings, when a dream is turning to a reality. It is a real pleasure to look back and to express gratitude to all those whom without their help, encouragements and support this thesis have never been possible.

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☺*Sunil Mundra*
Longyearbyen (78 °N); September, 2015

My PhD journey was impossible without this cute little plant Bistorta vivipara (Harerug in norwegian), and associated fungal partners (Photos © - Sunil Mundra).

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LIST OF PAPERS

This thesis is based on the following four studies (publications and manuscripts), which are referred in the text by their Roman numerals:

I. **Mundra, S***, Halvorsen, R, Kauserud, H, Müller, E, Vik, U and 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.

II. **Mundra, S***, Bahram M, and Eidesen, PB. Alpine bistort (Bistorta vivipara) in edge habitats associates with fewer but different ectomycorrhizal fungal species: a comparative study of three contrasting soil environments in Svalbard (*Manuscript*).

III. **Mundra, S***, Halvorsen, R, Kauserud, H, Bahram M, Tedersoo, L, Elberling, B, Cooper, EJ, Eidesen, PB. Ectomycorrhizal and saprotrophic fungi respond differently to long-term experimentally increased snow depth in the High-Arctic archipelago Svalbard (*Manuscript ready for submission to Global Change Biology*).

IV. **Mundra, S***, Bahram, M, Tedersoo, L, Kauserud, H, Halvorsen, R, and Eidesen, PB. Temporal variation of *Bistorta vivipara*-associated ectomycorrhizal fungal communities in the High Arctic (*Manuscript under revision at Molecular Ecology*).

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SUMMARY

Fungi play crucial roles in decomposition, symbiotic interactions, and biogeochemical cycling in most terrestrial ecosystems, including the Arctic. Arctic species and ecosystems are, by nature, highly evolved, with function and timing of seasonal events and finely tuned to their habitat. However, ongoing climate change is having detrimental effects on this balance. Therefore, an understanding of the fungal biodiversity of today's ecosystems is urgently needed. The main objective of this work was to investigate fungal richness and community structure, and their drivers at different spatial and temporal scales in the High Arctic region, using high-throughput sequencing of the internal transcriber spacer (ITS) region. In three studies (I, II, IV), fungal communities of *Bistorta vivipara* (ectomycorrhizal "ECM" plant species with wide spread distribution in the Arctic) roots were explored, in samples collected from natural Arctic tundra environment. In one study (III), soil samples were collected from a winter snow manipulation experimental site. All study sites were located on Svalbard. Sampling was performed from fine (I; centimetre) to broad (II; kilometre) spatial; and fine (III; weekly) to broad (IV; monthly, seasonally) temporal scales. I found that fungal community structure varies both spatially and temporally and patterns are typically scale-dependent. Structural patterns were weaker at fine scales than at broad scales of space and time. At broad scales, community structure variation was related to variation in environmental conditions (temperature, moisture, soil properties etc.); however, a large proportion of community variation remained unexplained at all scales. Root-associated fungal richness showed a typical species-area relationship, and was related to root size per host plant, geographical span of sampling. Fungal richness was also related to environmental factors, e.g. lower richness was found in sites with soil conditions characterized as edge habitats for the host plant, and experimentally increased snow depth clearly influence the fungal richness. Furthermore, I found that temporally fluctuating environmental and weather conditions significantly influence fungal richness, and that increase in winter. ECM fungal genera *Tomentella*, *Cortinarius* and *Inocybe* were highly frequent both in roots and soil; in addition, saprotrophic genera *Mortierella* was common in soil. In conclusion, the spatial structure of fungal communities is influenced by environmental filtering over broad spatial scales, whereas stochastic processes are more important on finer scales. Temporal variations in weather and environmental conditions are important determinant of community structural pattern both at fine and broad (growing season versus winter) temporal scale. However,

given the large residuals it is very difficult to pin-point the key drivers of community variations operating at different scales.

INTRODUCTION

General background

The kingdom fungi includes moulds, mushrooms, lichens, rusts, smuts and yeasts and is one of the most diverse groups of eukaryotic microorganisms that play fundamental ecological roles. Fungi are important belowground components in all terrestrial ecosystems; e.g., they drive nutrient cycling and influence aboveground biomass production through their roles as mutualistic organisms (e.g., mycorrhizae, endophytes and lichens), decomposers and pathogens (Smith & Read 2010). Approximately 80% of all known terrestrial plants form associations with mycorrhizal fungi (Trappe 1987). Fungi are also an important food resource for micro-arthropods such as Oribatid mites (Schneider *et al.* 2005), Collembola (Scheu & Simmerling 2004) or Enchytraeid worms (Hedlund & Augustsson 1995). According to Kirk *et al.* (2008) there are ~98,128 species of fungi described to date (accounting for synonyms). However, previous efforts by Hawksworth (1991) estimated the number of fungal species to reach ~1.5 million. In fact, recent estimates based on new sequencing methods predict the existence of over ~5 million of fungal species in the world (Blackwell 2011). That being true, we have discovered to date a mere ~2% of all extant fungal species. Therefore, we still have a limited understanding on fungal taxonomy and environmental sampling for this group, and there is a pressing need to re-evaluate fungal diversity (Bass & Richards 2011).

Ecology studies the interactions among organisms and between organisms and their environment. In addition, the main goal of community ecology is to identify the mechanisms defining the structure of ecological communities and their variation in space and time. To this end, the fundamental principle underlying existing biodiversity patterns, known as the distance decay of similarity, defines how the level of similarity among communities decrease with the increase in geographical and temporal scales (Gaston & Blackburn 1999; Nekola & White 1999; Whittaker *et al.* 2001). Thus, patterns change across spatial and temporal scales, and specific patterns are usually determined by multiple processes working at different scales and/or are scale-dependant. Therefore, species diversity could only be fully understood through studies incorporating observations from different temporal and spatial scales and testing multiple hypotheses specifically generated to assess species diversity. Knowledge on spatio-temporal variations in fungal richness and community structure is crucial to understand the structure and dynamics of all terrestrial ecosystems; however, obtaining this type of information is far from simple (Fierer 2008).

In recent years, researchers have shown an interest in understanding how variations in geographical and temporal distances influence fungal community composition in different terrestrial ecosystems. Thus, fungal communities are known to be influenced by multiple patterns and processes and to be strongly scale-dependent (Vik 2014); in addition, previous studies have also documented that community structure varies with space (Bahram *et al.* 2012; Bahram *et al.* 2013; Põlme *et al.* 2013; Tedersoo *et al.* 2014; Davison *et al.* 2015) and time (Buée *et al.* 2005; Izzo *et al.* 2005; Koide *et al.* 2007; Smith *et al.* 2007; Davey *et al.* 2012; Voříšková *et al.* 2014; Vargas-Gastélum *et al.* 2015). Such variations in fungal communities are caused not only by niche-based processes such as host species and taxa, soil type, nutrient availability or changes in environmental conditions with increasing distance (Lilleskov *et al.* 2002; Toljander *et al.* 2006; Ishida *et al.* 2007; Peay *et al.* 2010a; Kjøller *et al.* 2012; Tedersoo *et al.* 2013), but also by stochastic processes mainly caused by dispersal limitations (Hubbell 2001; Cottenie 2005; Peay *et al.* 2010b; Peay *et al.* 2012; Peay & Bruns 2014). However, despite the importance of spatial and temporal variations in defining patterns of biological diversity, these patterns and their drivers are poorly known for High Arctic fungal communities (Gardes & Dahlberg 1996).

The Arctic environment is characterised by cold winters and cool summers, with an average air temperature for the warmest month (July) below 10 °C. Low precipitation, low moisture conditions, short growing seasons, wind exposure, presence of continuous permafrost and soil movement caused by freeze-thaw cycles provide an unfavourable condition for Arctic tundra biotic communities (Chapin & Shaver 1981; Chapin & Körner 1995; Callaghan *et al.* 2005). Additionally, periglacial processes also influence the vegetation and soil conditions at a distances below 1 m (Washburn 1980).

Even in such extreme Arctic conditions, fungi are ubiquitous in Arctic environments (Newsham *et al.* 2009; Geml *et al.* 2012; Timling & Taylor 2012) and are abundant in soil and plant tissues. Fungi have also been recorded from other Arctic habitats such as sea-water and sea-ice (Gunde-Cimerman *et al.* 2003; Kristiansen 2014), cryoconite holes, glacial environments (Säwström *et al.* 2002; Sonjak *et al.* 2006; Cameron *et al.* 2012) and permafrost (Ozerskaya *et al.* 2009; Kochkina *et al.* 2012; Bellemain *et al.* 2013). Fungi represent one of the most diverse groups of organisms in the Arctic (Dahlberg *et al.* 2013). Fungal species from all major phyla have been recorded in this region (Wallenstein *et al.* 2007; Geml *et al.* 2012), being also represented in less extreme environments. According to Dahlberg *et al.* (2013), approximately 4,350 fungal species have been described from the Arctic, although the total number may well exceed 13,000 due to lack of data, possibly

because of their cryptic nature. Similar to other elements of terrestrial ecosystems, fungi play crucial ecological roles in the Arctic, including recycling soil organic matter (saprotrophs), acting as important plant and animal pathogens (biotrophs), transferring nutrients and water from the soil to their hosts, particularly through their association with plants as mycorrhiza (symbiotrophs) and enhancing plant performance (Gardes & Dahlberg 1996; Ludley & Robinson 2008; Newsham 2011; Timling & Taylor 2012).

Mycorrhizal symbiosis is mutualistic relationship, where fungi assist plants' uptake of nutrients and water from soil and receive C from the host plant in return. They have pivotal roles in Arctic terrestrial ecosystem where low water and poor nutrient availability limit plant growth and productivity (Gardes & Dahlberg 1996; Timling & Taylor 2012). Depending upon the host and mode of root infection there major type of mycorrhizal association (arbuscular - fungi produce arbuscules, hyphae, and vesicles within root cortex cells; ericoid – fungi form coils of hyphae within thin hair roots of the Ericaceae plants; and ectomycorrhiza (ECM) - fungi form a mantle around roots and a Hartig net between root cells), possibly exist in Arctic (Gardes & Dahlberg 1996; Newsham *et al.* 2009). ECM fungi is of particular interest in Arctic due to their ability form association with many wide spread Arctic plant species belonging to genera such as *Bistorta, Betula, Dryas and Salix* (Hesselmann 1900; Väre *et al.* 1992). ECM fungi not only provides majority of the nitrogen (N) to Arctic plants (Hobbie & Hobbie 2006; Hobbie *et al.* 2009), but also they have ability to survive at lower temperatures than their host roots (Lehto *et al.* 2008; Korhonen *et al.* 2013). These ecosystem services are fundamental not only for primary production, but for the long-term functioning of the Arctic ecosystem.

The Arctic region has become warmer over the past century (Kaufman *et al.* 2009) and climate models predict a continuous warming following the trend in anthropogenic carbon and greenhouse emissions (Moritz *et al.* 2002; A.C.I.A. 2005). This warmer climate is also expected to influence air and surface temperatures and summer and winter precipitation patterns. Many climate models predict an increase in precipitation of more than 50% over the levels recorded in 2006-2014, in particular as snow in winter (A.C.I.A. 2005; IPCC 2013; Bintanja & Selten 2014). Increased snow accumulation can also enhance soil temperature and act as a temperature buffer (Semenchuk *et al.* 2013). The large carbon (C) pool in permafrost soil is very sensitive to such climatic fluctuations (Davidson & Janssens 2006; Elberling *et al.* 2013), and increased temperatures may release large amounts of stored C through increased soil respiration (Karhu *et al.* 2014). This turnover of soil C also threatens the stability of the Arctic C pool. To this end, several studies have already shown

how Arctic tundra vegetation has changed in response to recent climate warming (Chapin *et al.* 1995; Sturm *et al.* 2001; Walker *et al.* 2006; Elmendorf *et al.* 2012; Sistla *et al.* 2013; Rumpf *et al.* 2014).

Despite the importance of fungi in Arctic ecosystems, our knowledge on how fungi respond to on-going climatic change is still scarce, in particular regarding their response to warmer soil conditions during winter. There is a growing body of literature investigating the effect of summer warming, suggesting significant changes in fungal richness and composition (Clemmensen *et al.* 2006; Deslippe *et al.* 2011; Deslippe & Simard 2011; Deslippe *et al.* 2012; Morgado *et al.* 2014; Geml *et al.* 2015). More recently, Semenova *et al.* (2014) reported an increase in proportion of saprotrophic fungi, both in dry and moist tundra, due to an increase in litter accumulation during summer in plots subjected to warming (Wahren *et al.* 2005; Walker *et al.* 2006). Warming-induced changes in soil fungal community composition may cause substantial changes in fungal decomposer activity in mineral soils (Timling & Taylor 2012; Sistla *et al.* 2013), and an increase in C utilization and C losses to the atmosphere (Rinnan & Bååth 2009).

Arctic conditions are in general logistically challenging, and a short growing season and irregular fruiting patterns are major limitations to understand the distribution and ecological patterns of Arctic fungi. Earlier Arctic fungal surveys were based on the collection of fruiting bodies (mushrooms), microscopy of roots and fungal culture isolation, which allowed detecting only a small fraction of total Arctic fungal diversity (Petersen 1977; Väre *et al.* 1992; Gulden & Torkelsen 1996). The applications of molecular approaches, on the other hand, are essential to untie the black box of Arctic fungal ecology (Gardes & Dahlberg 1996; Horton & Bruns 2001; Peay 2014). Thus, High-Throughput Sequencing (HTS) is a powerful alternative to traditional identification techniques, as it enables us to identify the presence of hundreds of co-existing species in relatively small environmental samples. More importantly, this technique allows us analysing a large number of samples whilst providing a considerable sequence depth per sample. The development of HTS methods has revolutionized fungal ecology (Lindahl *et al.* 2013), capturing the enormous fungal diversity that can be present in each individual environmental sample (Buée *et al.* 2009; Jumpponen & Jones 2009; Öpik *et al.* 2009; Amend *et al.* 2010; Jumpponen *et al.* 2010; Tedersoo *et al.* 2010; Dumbrell *et al.* 2011; Davey *et al.* 2012; Kauserud *et al.* 2012; Clemmensen *et al.* 2013; Schmidt *et al.* 2013; Talbot *et al.* 2014; Tedersoo *et al.* 2014; Sterkenburg *et al.* 2015).

Arctic species and ecosystems are, by nature, highly evolved in function and finely tuned within their habitat and timing of seasonal events (Hodkinson *et al.* 2001; Hodkinson & Coulson 2004). This fine-tuned balance is currently being disturbed both at the spatial and temporal scales due to climate change, increasing the urgency in the need to understand and comprehensively describe current Arctic fungal diversity patterns. This gap in Arctic fungal diversity knowledge, in particular regarding spatial and temporal patterns, and the level of sensitivity of Arctic biodiversity to climate change, inspired me to use HTS methods to identify the spatial and temporal variation of High Arctic fungi and the underlying mechanisms determining these patterns.

Pre-existing knowledge on spatial and temporal variation in High Arctic fungi

Existing literature regarding the main topics of this thesis is scarce; however, there are some examples of studies on the spatial structure of Arctic fungi at different scales. At the fine spatial scale (3 × 3 m), Botnen *et al.* (2014) found that root-associated fungal communities lack spatial structure in natural Arctic tundra environments. In contrast to natural tundra environments, patterned ground features (periglacial characteristics) significantly affect soil fungal community structure to an spatial scale down to a meter (Timling *et al.* 2014). At broad scale (kilometre), root-associated fungal community were similar within sites and regions, suggesting a mechanism of environmental filtering for structuring communities (Blaalid *et al.* 2014). In another study, comparing alpine sites (Norway) and a High Arctic site (Svalbard), Bjorbækmo *et al.* (2010) found a weak geographical structure in *Dryas octopetala* root-associated fungal communities at a broad spatial scale; however, they found high spatial heterogeneity within each locality. Moving towards a more broader spatial scale over the North American Arctic, encompassing the five bioclimatic zones, Timling *et al.* (2012) and Timling *et al.* (2014) found that root-associated and soil fungal community structure change gradually among bioclimatic zones and was affected by geology, soil properties and vegetation.

When comparing fungal richness at the global level, this tends to decrease with the increase in latitude (Tedersoo *et al.* 2012; Tedersoo *et al.* 2014). However, this pattern did not apply for higher latitudes in the Northern hemisphere (Bjorbækmo *et al.* 2010; Timling *et al.* 2012; Timling *et al.* 2014). Within the Svalbard archipelago (encompassing three bioclimatic zones, A, B and C), Blaalid *et al.* (2014) found a slight decrease in per-plant OTU richness with the increase in latitude and suggested that Arctic fungi were not subjected to dispersal limitations in this region (Geml *et al.* 2012). Compared to broad

spatial scales, environmental conditions are highly heterogeneous at the fine scale, possibly leading to a reduced stability of fungal communities (Izzo *et al.* 2005), which could consequently affect their spatial patterns (Pickles *et al.* 2009; Wolfe *et al.* 2009). Due to the importance of environmental filtering in shaping Arctic fungal community structure, it is essential to identify the environmental filters acting across different spatial scales (Timling & Taylor 2012; Blaalid *et al.* 2014).

Compared to spatial structure, much less is known about the temporal structure of fungal communities (Bahram *et al.* 2015), especially in the Arctic (Timling & Taylor 2012). Available literature on temporal variation in Arctic fungal communities suggests that fungal communities are relatively stable at higher taxonomical level, with some variation at the order level (Cantharellales and Aphyllophorales) within the growing season (Wallenstein *et al.* 2007; Deslippe *et al.* 2012). Within higher taxonomic levels, some species or genera that may also be active only under a smaller range of environmental conditions therefore vary temporally. In the studies mentioned above, sampling frequency was low and they were based on automated ribosomal intergenic spacer analysis (ARISA) and traditional Sanger sequencing. Sanger sequencing from environmental samples, may provide overall taxonomic diversity information, but it is unable to capture complete coverage therefore, is insufficient in describing community complexities.

Along a temporal succession gradient (~72 to ~10 000 years), root-associated fungi show a directional non-replacement shift in the Arctic (Davey *et al.* 2015), implying that fugal communities change at broad temporal scale. Therefore, it is essential to understand the possible influence of climate warming and changes in seasonality on Arctic ecosystems (A.C.I.A. 2005). Changes in climatic conditions may affect fruiting patterns (Kauserud *et al.* 2010) and spore production (Kauserud *et al.* 2011), which could subsequently influence the temporal variation of fungal community structure and richness.

Most Arctic fungal studies have been conducted during the snow free growing-season, assuming that fungal activity is restricted or does not occur at low temperatures in frozen soils during the winter (Tibbett & Cairney 2007). However, evidence suggests that (a) snow cover is an important factor driving the seasonal variation in fungal communities in cold environments (Zinger *et al.* 2009); (b) certain cold adapted fungi are able to grow and survive at low temperatures (Robinson 2001; Lehto *et al.* 2008; Korhonen *et al.* 2013); (c) a significant amount of decomposition takes place in winter under the snow (Schmidt & Lipson 2004); and (d) microbial biomass peaks in winter under the snow (Lipson *et al.* 2002; Kuhnert *et al.* 2012) and this biomass is dominated by fungi (Schadt *et al.* 2003;

Nemergut *et al.* 2005; Buckeridge *et al.* 2013). Seasonal variation in fungi can for instance drive changes in the patterns of nutrient cycling (Schadt *et al.* 2003; Schimel *et al.* 2007). Therefore, knowledge on temporal variation in fungal communities, and how this relates to the variation in environmental conditions, is important to understand soil nutrient dynamics in the Arctic tundra.

Biases associated with HTS-based analyses of fungal communities

HTS provides an opportunity to investigate deeper layers of the microbial communities and allows to identify a larger number of species with a less biased qualitative picture of the community composition compared to other molecular techniques (Sogin *et al.* 2006; Öpik *et al.* 2009; Lindahl *et al.* 2013). These platforms include Roche 454, Illumina, IonTorrent, PacBio, Helicos, SOLiD and NANOPORE. All these systems rely on a complex combination of chemistry and computing capabilities and have their own drawbacks and benefits depending on the type of study. In addition, constant developments in HTS technology are a cause of major concern, as the old versions of the technique can potentially become obsolete with the advent of a new one. In 2013, Roche announced that 454 platforms will not be supported in the near future. The surge of cheaper and >300 bp paired-end (PE) sequencing techniques developed by MiSeq along with the >8000 bp reads from PacBio essentially suppressed the use of 454 technology. Following this technological development, I also switched from 454 (I) to Illumina (II-IV) during the progress of this work.

The constant development in HTS technology means that the newer methods outperform earlier approaches in terms of resolution and magnitude, providing unprecedented insights into fungal community ecology. However, without a deep understanding on the possible methodological biases, limitations related to the type of markers used or other bioinformatics challenges, large-scale sequencing risks yielding artificial results and misleading conclusions. Thus, it is possible to introduce errors in fungal community analyses from field sampling via laboratory procedures, enzyme and primer selection, tag switching, bioinformatics analyses and data interpretation (Carlsen *et al.* 2012; Lindahl *et al.* 2013; Oliver *et al.* 2015; Philippe *et al.* 2015; Schnell *et al.* 2015). However, HTS-induced errors could be reduced, to some extent, with knowledge currently available following the incorporation of fungi into FUNGuild (Nguyen *et al.* 2015), SCATA (http://scata.mykopat.slu.se), PIPITS (Gweon *et al.* 2015), QIIME (Caporaso *et al.* 2010) and MOTHUR (Schloss *et al.* 2009) platform tutorials and in the guideline and protocol for HTS

data analysis (Huse *et al.* 2010; Nilsson *et al.* 2011; Lindahl *et al.* 2013; Bálint *et al.* 2014; Nguyen *et al.* 2014; Hart *et al.* 2015; Tedersoo *et al.* 2015). In addition, errors could be further limited through the use of a well curated ITS database (Kõljalg *et al.* 2013),using the ITS sequence dataset for reference-based chimera analysis (Nilsson *et al.* 2015), the application of robust clustering algorithms (Huse *et al.* 2010; Kunin *et al.* 2010; Edgar *et al.* 2011) and using robust fungal specific primer targeting ITS2 region (Ihrmark *et al.* 2012) and software for detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi (Nilsson *et al.* 2010; Bengtsson-Palme *et al.* 2013). Increasing sequencing depth per sample (Smith & Peay 2014) and using repeated PCR reactions with different annealing temperatures can also increase the recovery of fungal diversity (Schmidt *et al.* 2013). Thus, through the combined application of the approaches and guidelines mentioned above, most biases and limitations associated with HTS are likely avoided. However, the use of a 'mock community' is highly recommended as a positive control in HTS analysis (Nguyen *et al.* 2014).

The model study plant system: Bistorta vivipara

Bistorta vivipara (L.) Delarbre (Polygonaceae; syn.: *Polygonum viviparum*) is an ectomycorrhizal, polyploid (high and variable chromosome numbers (2n = c.77–132; c.7x–12x), perennial herbaceous (2.5–24 cm high) plant species, with a wide distribution in circumpolar Arctic and Alpine habitats (Hultén 1968; Aiken *et al.* 2007). Earlier findings have shown that pH and nutrient levels are particularly important for average performance and density of *B. vivipara* (Wookey *et al.* 1994; Totland & Nyléhn 1998; Bills *et al.* 2015). This plant species was used as study plant in three studies (I, II, IV).

Bistorta vivipara have a mixed reproduction system enabling both sexual and asexual reproduction; bulbils are typically borne in basal regions and flowers are borne distally within an inflorescence (Fig. 1a). Flowers rarely produce viable seeds and asexual reproduction is normally by the bulbils, which disperse from the inflorescence, form roots, and establish new physiologically independent plants (Diggle 1997). Bulbils (also termed as brood tubers) are small bulb-like structures, rich in starch, and preferred food for Rock Ptarmigan - *Lagopus mutus* (Moss & Parkinson 1975), barnacle goose - *Branta leucopsis* (Kuijper *et al.* 2006), and reindeer (Lindwall *et al.* 2013). The main axis of *B. vivipara* is an unbranched rhizome, grows plagiotropically, 3–4 cm below the soil surface having the roots and apical meristems are attached.

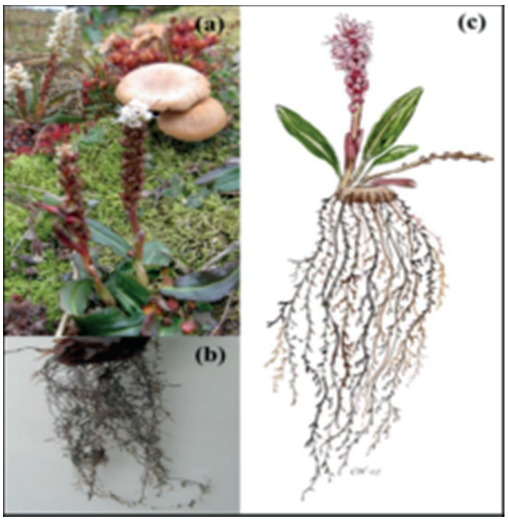

Figure 1. *Bistorta vivipara (a) above ground part of the plant includes the inflorescence (flower and bulbils) (b) washed root system where roots are attached to the rhizome of the plant and showing extensive ECM root tips and (c) hand drawing of the plant showing* both above- and below-ground part. (Photos © - Pernille Bronken Eidesen (a), Sunil Mundra (b), Cecilia Helmerson (c)).

Using microscopy, morphological and culture based technique it has been shown that *B. vivipara* form an ECM symbiosis with fungi (Hesselmann 1900; Read & Haselwandter 1981; Massicotte *et al.* 1998). The small and compact root system of *B. vivipara* (Fig. 1b, 1c) allows to study whole root fungal community (Blaalid *et al.* 2012; Kauserud *et al.* 2012), and makes it an excellent model plant with high level of reproducibility in community profile. Although, *B. vivipara* is ECM plant, other fungal groups such as latent saprobic, pathogenic and dark septate fungi fungi were also observed on roots (Newsham *et al.* 2009; Blaalid *et al.* 2012; Yao *et al.* 2013; Blaalid *et al.* 2014; Botnen *et al.* 2014).

OBJECTIVES

The main objective of the thesis was to investigate the patterns and underlying processes of fungal richness and community structure at different spatial and temporal scales in the High Arctic, using Svalbard as study location.

The main objective was approached through four studies addressing three major research questions:

- How do root-associated fungal richness and communities vary spatially: from fine scale (centimetre; study I and II) within the same habitat to broad scale (kilometre; study II) among habitats; and what are their drivers?
- How do root-associated fungal richness and communities vary temporally: from fine scale (weekly; study III) to broad scale (monthly and seasonally; study IV); and what are their drivers?
- How do nutritionally edge habitats of the host plant contribute to overall Arctic fungal diversity (study II); and what are the core Arctic fungal taxa (study I-IV)?

MATERIAL AND METHODS

Study sites and sampling design

Sampling for all four studies were carried out in a High Arctic archipelago, Svalbard (ranging from 74-81°N and 10-35°E; Fig. 2a). Svalbard is often described as an undisturbed Arctic environment and falls within the zone of continuous permafrost. Periglacial and permafrost-related terrain features are widespread in areas that are not covered by glaciers. The geology of Svalbard is also highly diverse, and various bedrocks further influence the soil properties (Harland 1998). Three of the five Arctic bioclimatic (BC) zones are represented in Svalbard (Elvebakk 1999; Walker *et al.* 2005): the middle Arctic tundra zone (BC zone C; mean temperature for the warmest month: 5-7 °C); the northern Arctic tundra zone (BC zone B; 3-5 °C) and the Arctic polar desert zone (BC zone A; 3°C). The zones are recognized by the difference of the plant communities, known as zonal vegetation types (defined by various plant and moss species and their coverage). Sampling design differed for each study based on the specific ecological question (see each article for the details). Sampling for study I (fungal community structure at fine spatial scale) was performed in *Dryas* heath in front of the Midtre Lovénbreen glacier (Ny Ålesund; Fig. 2b). For two studies (II - fungal community structure at broad spatial scale, IV - temporal variation in Arctic fungi) samples were also collected in *Dryas* heath, but from a different location (Isdammen; Fig. 2c). Additional samples for study II were collected from a high productive bird-cliff (Vestpynten) and a low productive mine habitats (Bjørndallen; Fig. 2c). Sampling for study III (effect of increased snow depth on Arctic fungi) was performed at a mesic meadow site located in the Adventdalen valley (Longyearbyen). Study III differed from the others in two ways; firstly, sampling plots

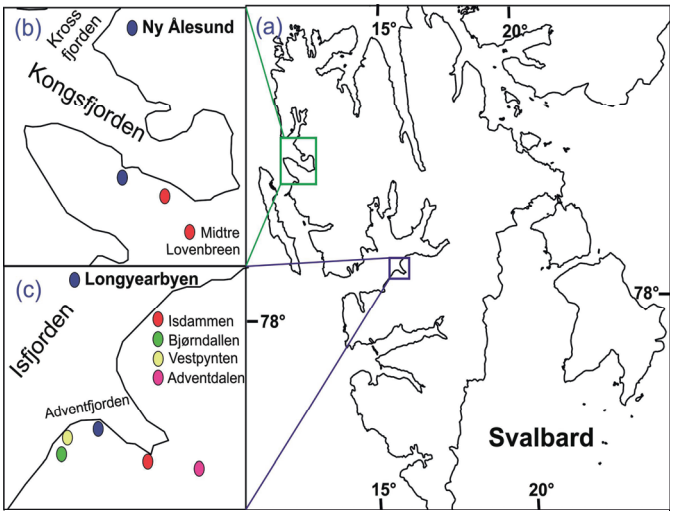

Figure 2. *An outline map of (a) Svalbard, (b) Ny Ålesund and (c) Longyearbyen, showing different study sites.*

were placed in an experimental set-up rather than along natural gradients. The experimental set up constituted of six snow fences (used to enhance winter snow accumulation) and control plots established by Elisabeth Cooper in 2006 (Cooper *et al.* 2011). A small sampling plot (0.5 × 0.5 m) was established in both deeper snow area of fence (~ 5 away from fence) and control area. Secondly, fungi in soil samples were analysed rather than fungi associated with *B.* vivipara roots. For other three studies (I, II, IV), plants with their whole root system were excavated and treated as described in study I. Relevant abiotic and biotic variables were measured for each study (for details see individual study).

Molecular analysis

In three studies (I, II, IV) DNA was extracted from the entire plant roots using a modified CTAB extraction protocol (Murray & Thompson 1980). In study III, total soil genomic DNA was extracted using PowerSoil® DNA Isolation Kit (MO-BIO Laboratories, CA, USA), according to the manufacturer's protocol. The extracted DNA was further purified using the E.Z.N.A soil DNA kit (Omega Biotek, USA) following the manufacturer's protocol. The 454 sequencing technique was used in study I; fungal specific primers ITS1F and ITS4 (White *et al.* 1990; Gardes & Bruns 1993) were used in the first step, whereas the ITS5 and ITS2 fusion primers (White *et al.* 1990) were used in the nested step that targeted the internal transcriber space (ITS) 1 region of the nuclear ribosomal rDNA repeat. In other three studies (II-IV) Illumina PE (300 × 2) sequencing was employed; forward primers fITS7a (Ihrmark *et al.* 2012) and reverse primer ITS4 (White *et al.* 1990) were used to amplify ITS2 region. Bioinformatic treatment of 454 and Illumina data differed slightly. Reads with length <200 bp and >550 bp, homopolymers exceeding 8 bp, ambiguous base call >0, and more than one mismatch in the forward primer sequence, were removed from the data set, using split_library.py function implemented in QIIME v. 1.8.0 (Caporaso *et al.* 2010). Quality filtered reads were exercised for de-novo chimera checking using usearch61 algorithm (Edgar 2010). Non-chimeric reads were clustered into Operational Taxonomic Units (OTUs) at 97% similarity threshold using the uclust algorithm and the most abundant sequence of each cluster was designated as representative sequence (Edgar, 2010). Clusters represented by <5 sequences were discarded as likely sequencing errors (Nguyen *et al.* 2014). Representative sequence of each cluster was subjected to BLASTn search against the quality-checked UNITE+INSD fungal ITS sequence database, containing both identified and unidentified sequences (Kõljalg *et al.* 2005; Kõljalg *et al.* 2013).

Statistical analysis

Most of the statistical analyses were carried out in R (R Core Development Team 2014). Detrended correspondence analysis "DCA" (Hill & Gauch 1980) and global nonmetric multidimensional scaling "GNMDS" (Kruskal 1964; Kruskal *et al.* 1973), were applied in parallel to describe patterns of variation in fungal OTU composition (I-IV; for more details see I). Assessment of similarity between GNMDS ordinations was made by Procrustes analysis (Oksanen *et al.* 2013). Accepted GNMDS solutions were rotated to principal components and the rotated axes were rescaled to half-change (H.C.) units by the *postMDS()* procedure in the *vegan* package. DCA ordinations were run with default options. All ordinations were inspected for known artefacts such as arch effects, tongue effects and strong outliers (Økland 1990). A reliable gradient structure was inferred if similar results were obtained by the use of the two methods (GNMDS and DCA) and no obvious artefacts were observed (Økland 1996). Similarity of ordinations was evaluated by calculating Kendall's rank correlation coefficient (τ) between pairs of DCA and GNMDS axes (Kendall 1938). Axes were considered similar if $|\tau| > 0.4$ (Liu *et al.* 2008). Interpretation was performed by calculation of τ between GNMDS axes and each explanatory variable, and by the use of the *envfit* function in *vegan* package, in which each explanatory variable (biotic and abiotic) is separately regressed on GNMDS axes 1 and 2 by linear regression analysis. Additionally, multivariate permutational analysis of variance "PERMANOVA" as implemented in the Adonis function of the package *vegan* was used to test the interaction terms (III, IV). Empirical semi-variance analyses, as implemented in the R package *geoR* (I) was used to describe the spatial patterns. Mantel test was performed to assess the correlation between two different distance matrices (II, IV). Temporal patterns of fungal communities were also investigated using partial canonical correspondence analysis "CCA" (ter Braak 1986), in which the effects of all spatial eigenvectors were partialled out from the constrained ordination.

Generalized Linear Model (GLM, I), ANOVA (II), Generalized Linear Mixed Models (GLMM) fit by maximum likelihood (III) and Covariate ANOVA (IV) were used to test the variation in fungal species richness in relation to factor and vector variables. Bonferroni correction of p-value was used at each step in the forward selection procedure (Blanchet *et al.* 2008) to prevent bias due to multiple testing (Legendre & Legendre 2012). Accumulation curve for OTUs and sampling effort were calculated following Ugland *et al.* (2003) protocol and shared species analysis was performed using EstimateS (Colwell 2013).

STUDIES

Study I. Fungal community structure at fine spatial scale

The aim of the study was to assess the structural relationship between the aboveground vegetation encompassing the host plant *B. vivipara* and the root-associated fungal community of this host plant, and to determine the possible biotic and abiotic drivers of fine-scale spatial patterns in richness and composition of root-associated fungi. The study site was located in at *Dryas* heath in front of the Midtre Lovénbreen glacier. Results showed that root-associated fungi lacked spatial structure at 0.3 × 3 m scale and were not affected by soil variables. A weak relationship between root-associated fungal communities and the cover of two ECM plants, *B. vivipara* and *Salix polaris*, was found, and richness increased with host root length and root weight. Results suggest that at fine spatial scales root-associated fungal communities are influenced by neighbouring ECM plants; but not by soil nutrients.

Study II. Fungal community structure at fine and broad spatial scale

Bistorta vivipara-associated fungal communities are highly heterogeneous over fine scale and environmental filtering operates at broad scales. Here, effect of environmental filtering on *B. vivipara*-associated ECM communities was further investigated by comparing core habitats with rarer edge habitats for the host. In this study, three sites consisting of one core habitat (*Dryas* heath) was compared with two edge habitats representing extremes in terms of nutrient-availability; a bird manured, nutrient-enriched site and a barren, nutrient-depleted mine tailings. Study site and associated soil conditions significantly affected community composition and richness of ECM fungi. ECM richness was overall lower in both edge habitats compared to core habitat. Community structure within each site was significantly influenced by spatial variables. Sharing of fungal species between habitats was low and communities of species poor edge habitats were poorly nestedness in species rich core habitat. Overall, species belonging to phylum Basidiomycota and genera *Tomentella*, *Cortinarius*, *Hebeloma* and *Cenococcum* were dominating, but within sites composition differ: stress tolerates genera *Hebeloma* and *Laccaria* were frequent in nutrient-poor site whereas functional competitor *Lactarius* and *Russula* were common in nutrient-rich site. In summary, environmental filtering do structure ECM community composition among different sites with strongly contrasting soil conditions, whereas stochastic spatial processes are more important within each site. Our results further show that rare edge habitats strongly contribute to the overall gamma-diversity of ECM fungi associated with *B. vivipara*.

Study III. Effect of increased snow depth on Arctic fungi

Aim of this study was to address the effect of experimentally increased snow depth on ECM and saprotrophic fungal species richness and community composition, over one growing season. The study site was located in the Svalbard. Soil samples were collected weekly from medio July to medio September in plots subjected to deep snow treatment and in control plots. In soil dominating fungal genera were *Tomentella*, *Cortinarius*, *Inocybe* and *Mortierella*. The richness of ECM fungi decreased while the richness of saprotrophic fungi increased in response to increased snow depth. Fungal richness significantly varied with time and peaked after a period with warmer and moister weather conditions; and observed temporal pattern of richness differ between deep snow and control treatment. A significant week snow treatment and sampling date effect was observed for saprotrophic fungal communities, but ECM fungal communities were not affected by snow treatment and time. Our results suggest that some fungal species are favoured while some are disfavoured by increased winter snow that may even go locally extinct.

Study IV. Temporal variation in Arctic fungi

Knowledge of temporal variation of ECM fungi, and the relationship of these patterns to environmental variables, is essential to understand energy and nutrient cycling in Arctic ecosystems. Roots of *B. vivipara* were sampled at 10 time intervals over two years; in the growing season (June, July and September) and in the winter (November and April). Sampling site was established in *Dryas* heath at Isdammen (Svalbard). ECM genera *Tomentella*, *Cortinarius*, *Inocybe*, *Hebeloma* and *Cenococcum* were temporally persistent, however, the species belonging to these genera varied in roots throughout both years and all seasons. Overall, ECM fungal richness seemed higher in winter, and species belonging to *Cortinarius*, *Serendipita* and *Sebacina* were more frequent in winter than during summer. Structure of ECM fungal communities was primarily affected by spatial factors. However, after accounting for spatial effects, significant seasonal variation was evident, and this variation showed some correspondence with seasonal changes in environmental conditions. Significant month × year interactions were observed both for fungal richness and community composition, indicating unpredictable between-year variation. Thus, to draw firm conclusions, replication over several years is needed. However, the available data indicate that arctic ECM richness and community structure differ between summer (growing season) and winter, possibly due to reduced activity of the core community, and addition of fungi adapted for winter conditions forming a winter-active fungal community.

DISCUSSION

The research in this thesis has increased our knowledge and understanding of how richness and community structure of High Arctic fungi vary through space and time, and how they are influenced by biotic and abiotic factors. The scale of sampling in this study ranged from fine to broad, both spatially (centimetre to kilometre) and temporally (weekly, monthly to seasonally), which covered a small amount of possible environmental variations existing in the Arctic terrestrial ecosystem. At fine both spatial and temporal scales, fungal communities show weak structural patterns; stochastic phenomena seem to be important for structuring communities on these scales. At broad spatial and temporal sampling scales, community structural pattern becomes clearer. Variation in environmental conditions (i.e. temperature, moisture and soil conditions) plays a major role in shaping the communities at these broad scales. It is noteworthy that fine spatial heterogeneity existing in Arctic fungal communities blurs their temporal patterns (Izzo *et al.* 2005). Compare to fungal communities drivers for fungal richness differs slightly. Root-associated fungal richness increases with an increase in root surface area and also with extending sampling geographic span, demonstrating the existence of a species-area relationship for fungal richness (Arrhenius 1921). Additionally, richness also varies among habitats with diverse soil conditions, suggesting importance of local environmental conditions. Across different temporal scales, moisture and temperature seems important factors causing variation in fungal richness patterns. Overall, it seems that community and richness patterns vary across different scales and that different factors drive the observed patterns. Therefore, it is difficult to assess the scale relevant for particular ecological theories, as ecological processes operate over a range of spatial and temporal scales (Bunnell & Huggard 1999; Pickles *et al.* 2009; Wolfe *et al.* 2009; Chave 2013; Brickhill *et al.* 2015). Multi-scale spatial and temporal studies covering a range of complex environmental gradients are likely to fill important gaps in our knowledge of compositional distributional patterns and the ecological processes operating on these scales (Halvorsen 2012). In this thesis, included studies were carried out at different spatial and temporal scales; hence variation of fungal community structure and richness across different scale can be addressed.

There has been much discussion about methodological limitations which may affect observations of fungal richness and communities (Huse *et al.* 2010; Nilsson *et al.* 2011; Lindahl *et al.* 2013; Bálint *et al.* 2014; Nguyen *et al.* 2014; Hart *et al.* 2015; Tedersoo *et al.* 2015). However, HTS methodologies are robust enough to capture ecological patterns in

fungal communities. Therefore, the Discussion here focuses on the ecological aspects of the results. Potential biases associated with applied HTS methods will not be discussed in the thesis, as a comprehensive literature review of this subject, and how to handle such data appropriately, are provided in the Introduction section. The main findings are briefly discussed below.

Fungal species richness and its drivers in the Arctic

Bistorta vivipara root-associated fungi were explored in three studies (I, II, IV), and soil fungi were investigated in another study (III). In one study (I) 454 sequencing was utilized with amplification of the ITS1 region, while in the other three studies (II, III, IV), Illumina Miseq sequencing was employed with amplification of the ITS2 region. However, considering that (a) sequence data were processed mostly using similar protocols and the same clustering threshold (97%) using QIIME (Caporaso *et al.* 2010; Edgar 2010); (b) 454 and Illumina sequencing data produce similar diversity estimates (Smith & Peay 2014); and (c) ITS1 and ITS2 region yield similar results (Blaalid *et al.* 2013; Monard *et al.* 2013); data across the studies can be compared. Among the three root-associated fungal studies, I found that overall fungal OTU (species) richness was highest in the temporal study over inter-annual scales (1165; IV), compared with both spatial studies (broad scale: 756; II; fine scale: 676; I; Fig. 3). In temporal study (IV), sampling was performed in different months across two year time period. Due to temporally variable environmental conditions in the Arctic, considerable temporal variation of fungi was expected, because tolerances to high temperature and low moisture differ among fungal species (Coleman *et al.* 1989; Robinson 2001; Talley *et al.* 2002). Therefore, different fungal species adapted to local environmental conditions exist at different time-point. Additionally, temporal turnover in host root biomass, the availability of root tips and local mortality of fungi may allow associations with different fungal species (Izzo *et al.* 2005). This overall leads higher fungal species richness with repeated sampling of the same site.

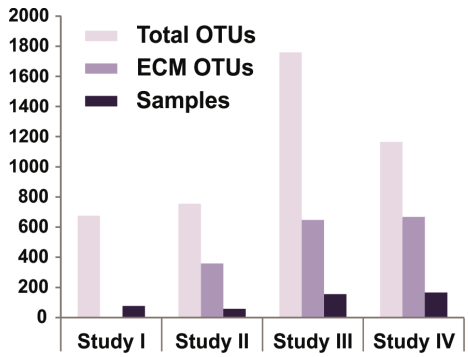

Figure 3. *Barplot showing the number of samples and total as well as ectomycorrhizal (ECM) fungal species in each of the study included in this thesis.*

The number of samples was fewer in broad spatial scale study (II) compared with fine spatial scale study (I), but sampling was performed in three localities differing in soil conditions. The existence of environmental filtering in Arctic habitats (Blaalid *et al.* 2014), creates a habitat-specific fungal species pool (II). Therefore sampling in habitat with different soil conditions will allow capturing more fungal species. I also found that within natural tundra habitat at fine spatial scales, size-related characteristics of *B. vivipara* roots were important determinants of fungal richness (I). Observed increase in fungal richness, when expanding the geographic spans of the sampling; and with increase in root size; suggesting a species-area relationship, where higher numbers of species are expected with increase in area being available for fungal colonization (Arrhenius 1921; Peay *et al.* 2007).

Across the studies, average total fungal species richness associated with roots of *B. vivipara* ranged from 56 – 71 in natural tundra habitat (I, II, IV). *Bistorta vivipara* root-associated fungal species richness observed here was similar to earlier HTS based studies (average 27 – 93 species per plant) from Arctic and Alpine areas (Yao *et al.* 2013; Blaalid *et al.* 2014; Botnen *et al.* 2014; Davey *et al.* 2015). In study I, and in the literature mentioned above, fungal species were not categorised functionally, and included ECM, saprotrophic, pathogenic, dark septate and lichen-forming fungi. However, a large proportion of species were ECM fungi. Similarly, in studies II and IV, I found that a major proportion of total fungal richness comprised of ECM fungi (27; II and 41; IV). ECM fungal richness recovered using HTS is higher than in earlier studies analysing *B. vivipara*-associated ECM fungi using root tip morphotyping and Sanger sequencing (Fontana 1977; Massicotte *et al.* 1998; Ronikier & Mleczko 2006; Mühlmann *et al.* 2008). This higher richness of ECM fungal species may indicate that host plant is able to associate with a range of fungal species having functional redundancy (Tedersoo *et al.* 2006; Courty *et al.* 2008; Bahram *et al.* 2011; Rineau & Courty 2011). Additionally, low host specificity of fungi may allow association with generalist fungi in the Arctic (Timling *et al.* 2012; Botnen *et al.* 2014). Association with multiple ECM species may provide resilience under variable environmental conditions (Druebert *et al.* 2009; Pena *et al.* 2010); enhances the host's productivity (Baxter & Dighton 2001; Jonsson *et al.* 2001; Wilkinson *et al.* 2012); and allow the plants to more rapidly and easily colonize newly available habitats (Botnen *et al.* 2014).

In contrast with natural tundra habitats, root-associated fungal richness was lower in nutritionally edge habitats of the *B. vivipara* plant. Sharing as well as nestedness of species between core tundra habitat and nutritionally edge habitats was also low (II). Lower richness in edge habitats are most likely related to environmental filtering processes, where fungal

species are influenced by low- and high-nutrient availability (Lilleskov *et al.* 2002). The low sharing of species among sites, and the weak nestedness of the edge habitat communities within the core habitat, suggests presence of a separate specialist fungal community in *B. vivipara* roots when growing in nutritionally marginal soil conditions. Following this strategy plant do not invest C for the less beneficial generalist fungal species (Kennedy 2010); and species with suitable functional traits for a given environment, and species that are able to survive under local environmental conditions are selected (Mayfield & Levine 2010).

Results showed that both total (139) and ECM (54) fungal species richness per sample was higher in soil (III) than in root (I, II, IV). Soil is inhabited by various generalist fungi with diverse functional roles in overall ecosystem functioning. Lower ECM fungal richness in *B. vivipara* roots suggests that they are a specific niche particularly for ECM fungi that facilitates specialist fungi (Kennedy 2010). Soil is a seed bank where both active and dormant fungal spores can persist (Lennon & Jones 2011), and it is therefore expected to be richer and more diverse. This may potentially result in higher ECM fungal richness in soil. In most of the analyses here, presence-absence data and/or Hellinger transformed data were used, with fungal biomass not being measured. Hence, variation in fungal abundance remains unknown. Nevertheless, ECM fungal abundance is expected to be higher on roots, due to their symbiotic nature and easy access for fungi to C from the host plant. Addressing this question requires careful sampling, in which all of the thin mycelia attached to host roots and extended into the surrounding soil are collected. Most of the current ECM fungal studies are relying on root-tips collection approach, which gives overall compositional pictures, but do not provide complete insights about the extent of their mycelial systems in soil; therefore knowledge of overall ECM mycelial dominance and their contribution to nutrient cycling remains scares (Genney *et al.* 2006; Anderson & Cairney 2007).

At fine temporal scales within the growing season, fungal richness significantly increased over the duration of a week, when higher precipitation and temperature were recorded (III). Over the broad temporal scale, fungal richness recorded for the winter seasons were similar and comparably high, whereas the richness level between growing-seasons differed (IV). Results from temporal studies suggest that in dry arctic environment, unfavourable environmental conditions such as poor soil moisture content, strongly limit the fungal richness. Therefore, higher precipitation-induced, greater soil moisture condition may trigger brief pulses of resource availability to fungi, and possibly can increase fungal richness, due to their differential water stress tolerance (Coleman *et al.* 1989; Talley *et al.*

2002). In arid desert ecosystems, it has been shown that fungal richness is affected directly by moisture (Zak *et al.* 1995) and/or indirectly by moisture-induced changes in soil chemistry, especially N (Fierer & Schimel 2002). I also found that variation in winter snow conditions and soil nutrient influence the fungal richness (II, III). Therefore, it is difficult to detangle the relative effect of weather and soil conditions on temporal variation of Arctic fungi.

The overall richness results show that fungal species richness is not distributed randomly over different spatial and temporal scales in the Arctic. Fungal richness increases with increasing root surface area and geographical distance of sampling. Additionally, a diverse species pool exists in the winter season and in habitats with diverse soil conditions. In their Arctic Biodiversity Assessment report, Dahlberg *et al.* (2013) suggest the existence of ~4350 fungal species in the entire Arctic. The study here, using HTS methods, detected distinct genetic groups representing over one third of this total Arctic fungal richness, but probably underestimated the number of species present in the sampled region, as rarefaction curves did not level off. This suggests that future studies should explore the fungal richness of Arctic ecosystems, since the existence of yet undiscovered groups is highly likely (Dahlberg *et al.* 2013). Habitats with diverse environmental and seasonally extreme conditions (winter season) are likely of great importance for the overall gamma diversity in the Arctic.

Considering the findings of spatio-temporal variation in fungal richness described above, it is recommended that future studies: (a) perform intensive spatial and temporal sampling; (b) collect detailed soil and weather metadata linked to each sample; and (c) consider potential methodological biases. This will strengthen the data gathered and should avoid misleading conclusions.

Core fungal taxa in Arctic

Across all studies, basidiomycetes were dominant, followed by ascomycetes (Fig. 4). At the phylum level, no clear compositional shift was observed across different spatial and temporal scales within natural tundra environment, indicating that representative taxonomic composition was recovered using HTS. Timling *et al.* (2014) and Bellemain *et al.* (2013) found a dominance of ascomycetes compared with basidiomycetes in Arctic patterned-ground soil and permafrost sediments. A possible explanation for this difference is the use of different molecular methodologies and the span of the sampling scale: for example, the latter study sampled 16,000–32,000 old permafrost soil. Within all root fungal studies (I, II, IV)

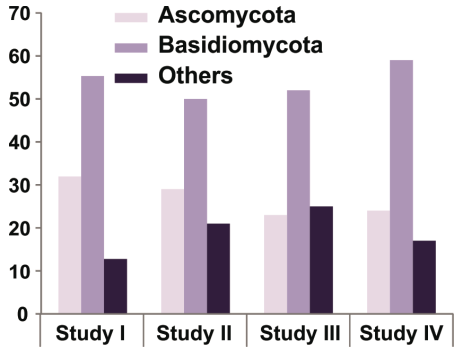

***Figure 4**. Phylum level distribution of total species detected in each of the study included in this thesis.*

typical ECM forming genera such as *Tomentella, Cortinarius, Inocybe*, *Russula*, *Hebeloma* and *Cenococcum* were dominant. In the soil study (III), the ECM forming genera *Tomentella*, *Cortinarius* and *Inocybe* were also dominating but thereafter saprotrophic fungal genus *Mortierella* was common. *Mortierella* is a cosmopolitan psychrophilic fungus occurring in soil and air (Bergero *et al.* 1999). Culture-based studies have shown *Mortierella* to be one of the most frequent genera in Arctic soil (Singh *et al.* 2012), and has the ability to degrade lignocellulose (Ruan *et al.* 2012).

Overall, the taxonomy results suggest that species belonging to the genera *Tomentella*, *Cortinarius* and *Inocybe* form a core Arctic fungal community, and probably have certain abilities that favour their survival in extreme Arctic environments. This is consistent with earlier reports showing dominance of these genera in Arctic soils (Geml *et al.* 2012; Morgado *et al.* 2014; Timling *et al.* 2014) and roots (Bjorbækmo *et al.* 2010; Timling *et al.* 2012; Blaalid *et al.* 2014; Botnen *et al.* 2014). The results here also corroborate a previous sporocarp collection study, which also observed a dominance of *Tomentella*, *Cortinarius* and *Inocybe* in the Arctic (Gardes & Dahlberg 1996). Species belonging to the genus *Tomentella* were common in nutritional edge habitats (II), and were not affected by increased snow depth conditions (III). This suggests adaptations of the genus to extreme and variable Arctic environmental conditions. The genus *Tomentella* includes species having contact, short distance and medium-distance type extramatrical mycelium morphology (Agerer 2001, 2006). Fungal species having longevity and higher mycelial spread, such as these *Tomentella*, are of particular importance for host plants, as they allow them to have a greater range of nutrient mobilization from organic compounds (Molina *et al.* 1992; Dahlberg & Stenlid 1995). Both *Cortinarius* and *Inocybe* are known to contain high species diversity, including several true arctic-alpine taxa (Gardes & Dahlberg 1996). The dominance of *Cortinarius* in Arctic tundra is possibly due to its medium-distance fringed exploration type and hydrophobic rhizomorphs, which provide better drought resistance (Agerer 2006; Lilleskov *et al.* 2011). The genus consists of symbiotic ECM fungi, but in stressful, nitrogen-limited Arctic environments it may decompose complex organic matter

for N mobilization (Bödeker *et al.* 2014). In study IV, I found that the species richness of *Cortinarius* was higher in winter, suggesting that the genus may possess survival strategies for winter conditions when C from the host plant is limiting.

Taxonomic composition was similar across all studies in natural tundra habitat (I-IV), but in non-optimal habitat of *B. vivipara* this fungal composition differ (II). As mentioned earlier that in edge habitats specialist fungal species exist, this was further supported by the difference in taxonomic distribution among sites (II). In the nutrient-poor metal rich mine tailing site, species belonging to *Laccaria* and *Hebeloma* were dominating. Both *Laccaria* and *Hebeloma* have differential expression of metallothioneins in response to heavy metals (copper and cadmium), therefore association with these ECM fungi probably aid survival and growth of ECM plants in areas contaminated by heavy metals (Ramesh *et al.* 2009; Reddy *et al.* 2014). It is also shown that species of *Hebeloma* can have high nutrient acquisition at low pH (Leprince & Quiquampoix 1996). In nutrient-rich bird-cliff site abundance of functional competitors *Lactarius* and *Russula* indicates their resistance to increased N level. Berg & Verhoef (1998) also found dominance of *Lactarius* at N saturated coniferous forest.

In all studies, a high proportion of OTUs remained taxonomically unclassified (average 29%), suggesting enormous fungal diversity with unidentified function and taxonomy persists in the Arctic (Schadt *et al.* 2003; Rosling *et al.* 2011). Sequencing of environmental DNA is a powerful tool to explore cryptic fungal diversity, but robust identification of fungal taxa using this technique is a huge challenge. I used well curated latest version of the UNITE reference sequences database for sequence identification (Kõljalg *et al.* 2005; Kõljalg *et al.* 2013), and seems there is necessity to include more reference sequences of known fungi, especially from the Arctic region. Additionally, suitable multi-locas approach for species identification can also give precise taxonomy annotations of fungal species (Gazis *et al.* 2011; Vrålstad 2011).

I used official barcode of fungi "ITS region" in the study (Schoch *et al.* 2012). This region consists of ITS1, conserved 5.8S region and ITS2 region. ITS1 region is more variable than ITS2 (Nilsson *et al.* 2008), which may result in some taxonomical differences (Mello *et al.* 2011). Therefore, the best option is to amplify whole ITS region for fungal identification, but widely used Illumina HTS technologies currently can only offer a read length of ~600 bp, and unable to cover whole ITS region. This suggests the need of cheaper sequencing technology providing more read length.

Fungal community structure and its drivers in the Arctic

In all studies, communities were explored using HTS, which allowed capturing rare species, which would otherwise have been underestimated using traditional methodologies. However, accumulation curves either did not approach asymptotes (I-IV). This suggests that (a) total fungal communities in any habitat and at each sampling time was not recovered, and that further extended sampling would reveal additional undiscovered species; (b) there is a high spatial (I, II) and temporal (III, IV) variation in communities, and so sampling more plots with higher sampling frequency might capture majority of present fungal community.

In agreement with Izzo *et al.* (2005), I also found that high spatial heterogeneity blurred the pattern of temporal variation in communities (III, IV), and therefore temporal sampling should be performed in a small area where soil and vegetation conditions are homogeneous. I found that root-associated fungal communities did not show any spatial autocorrelation above 0.3 m (I; i.e., neighbouring host plants separated by > 0.3 m have different communities). This heterogeneity may be explained by periglacial processes and fine scale topographic variation, which play a major role in increasing small-scale landscape heterogeneity in the Arctic (Washburn 1980; Stewart *et al.* 2014). Such features cause huge spatial heterogeneity in vegetation and soil conditions; consequently, winter snow cover can also vary at fine spatial scales, creating a local microhabitat. Snow cover can insulate the ground against extreme low air temperatures, increase the soil moisture level, alter soil nutrient availability and affect the duration of the growing season in the Arctic (Cooper 2014; Semenchuk *et al.* 2015). This fine scale variability in environmental conditions makes conditions to be more stressful for the underlying ecosystems and their component species. Plant roots and fungal mycelia experience highly variable conditions in terms of biotic and abiotic factors (mineral, nutrient, and water supply) even at temporal and spatial microscales. In such an extremely complex environment, heterogeneous fungal communities consisting of high numbers of micro-niches are expected. (Bruns 1995; Taylor 2002; Smith & Read 2010). However, a larger proportion of recovered fungal communities consisted of rare species, suggesting that fungal species co-exist rather than out-compete each other (Bruns 1995), and such co-existence may lead to reduced fungal community structure. Additionally, uneven sampling of temporally variable microniches may potentially influence the fungal community structural patterns.

In all studies, some associations between fungal community variation and biotic and abiotic environmental variables were found, but a considerable amount of variation remained unexplained, suggesting a high degree of randomness. This may be due to the

importance of competition, random extinction and colonization (Kennedy *et al.* 2009; Kennedy 2010; Koide *et al.* 2011; Kjøller *et al.* 2012; Pickles *et al.* 2012), which can enhance local variation in fungal community composition. The establishment of ECM is strongly limited by low spore germination in the absence of a host (Ishida *et al.* 2008), with limited ECM dispersal also enhancing spatial (Peay *et al.* 2012) and temporal (Peay & Bruns 2014) heterogeneity. In addition, unmeasured abiotic, and biotic factors such as belowground host C allocation, the release of spores, and/or recruitment of newly germinated mycelium during periods not suitable for vegetative growth and reproduction may affect fungal community structure (Bahram *et al.* 2015). Therefore, combined effects of all of these processes make it very hard to pin-point the key drivers of community variation operating at different spatial and temporal scales.

Although field-based ecological studies provide information on the role of specific processes in natural systems, it is difficult to generalize the results to systems other than those being directly studied. In a way field-based studies are similar to experiments, in that they involve setting up plots for sampling purposes; however, but it is very difficult to control many aspects of the system and some, e.g., temperature, precipitation, wind and soil composition are totally uncontrollable (Hairston 1989). Additionally, numerous biotic and abiotic interactions take place through space and time, making it difficult to assess the main drivers affecting biodiversity. Laboratory experiments performed in controlled environments can be useful to test different hypotheses generated by field research about the factors influencing fungal community ecology. Common garden/pot experiments under different soil conditions, where plants are generated from seed, may allow hypothesis testing about fungal community succession with space and time. In addition, the inoculation of known fungal species in such experiments can also add information about competitive interactions of fungi.

CONCLUDING REMARKS

The research in my thesis shows that fungal richness increases with an increase in root surface area and also with expanding sampling geographic span, demonstrating the existence of a species-area relationship. Additionally, results show that temporal changes in species richness can have multiple drivers - climatic and environmental conditions as well as duration of the studies. Species belonging to the genera *Tomentella*, *Cortinarius* and *Inocybe* form a core Arctic fungal community, and probably have certain abilities that favour their survival in extreme Arctic environments. I find that a high proportion of fungal species remains unclassified, which suggests that possibly, public sequence database lack proper reference sequences, and/or Arctic harbour enormous hidden fungal diversity which need to be explored (Dahlberg *et al.* 2013). The existence of specialist fungal species pool in edge habitats are of great importance for the overall diversity in the Arctic ecosystem and such habitat need to be included in large scale fungal biodiversity survey. Arctic fungal community structure varies both spatially as well as temporally; and structural patterns are typically scale-dependent. Ecological signals in the data (i.e., community variation along gradients) may be weaker at fine scales than at broad scales of space and time. I observe that high amount of community variations remains unexplained therefore, it very difficult to pin-point the key drivers of community variation operating at different spatial and temporal scales. High spatial heterogeneity in fungal communities mask the pattern of temporal variation in communities, therefore it is very important to consider contribution of both spatial and temporal variables in order to understand the underlying processes shaping fungal communities. I advocate the study of the underlying drivers behind temporal and spatial variation in fungal communities in order to facilitate understanding of how large scale environmental changes may affect biological communities in the future. The factors affecting spatial and temporal components of richness and communities affect not only the distribution and the number of fungal species, but also natural ecosystems and the ecosystem services that they provide.

FUTURE PERSPECTIVES – *the role of space and time in fungal community ecology*

- Fungal communities are highly heterogeneous even at very fine spatial scales in the Arctic. It is therefore important to conduct further studies in which interplant sampling distance is very low (< 0.3 m), in which sampling is at the root tip level, or where very minute soil samples at collected at very small (millimetre) spatial distances.
- To understand the true influence of large-scale geographical factors and possible influence of biogeographical parameters like dispersal and vicariance, sampling habitats should be as homogeneous among sites as possible. By investigating the same habitat type at several locations, the effect of local edaphic factors is minimised, and patterns created by other underlying or less influential factors may be revealed.
- In order to demonstrate effects of environmental and climatic changes on the seasonal dynamics of fungal community composition and richness, comparative studies over several years in different climatic regions are needed; and to account for annual variability in climate, long-term monitoring studies are suggested.
- Broad scale studies combining both spatial and temporal community variation are urgently required as global biodiversity is diminishing at an accelerated pace. It is critical to understand the underlying mechanisms of the factors affecting species richness and composition both locally and regionally in a broad range of terrestrial ecosystems.
- Soil nutritional level is often more limiting than temperature for Arctic vegetation, and based on the results presented here, soil conditions seems important for overall Arctic fungal diversity as well. Therefore, Arctic habitats with diverse condition need to be included in large-scale fungal biodiversity surveys and global initiatives such as earth microbiome projects (Gilbert *et al.* 2010) and national ecological observatory networks (Kampe *et al.* 2010).
- In order to determine overall biotic community dynamics, research is needed to move from a "who is there" to a "why are they there" and "what are they doing" perspective. Further research is required to detect any functional shifts related to spatial and temporal changes in fungal communities in the natural environment.

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PUBLICATIONS AND MANUSCRIPTS

- ***Study I***
- ***Study II***
- ***Study III***
- ***Study IV***